Vol. 50, No. 11, June, 1961

Russian Original Dated November, 1960

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

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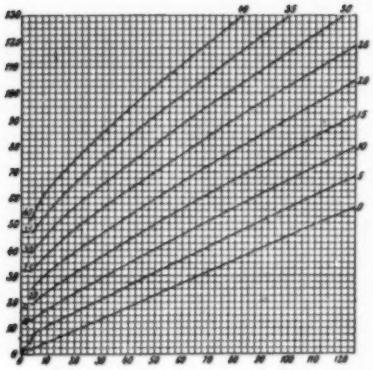

TABLES AND NOMOGRAMS OF HYDROCHEMICAL ANALYSIS

by I. Yu. Sokolov

Translated from Russian

Upon completion of analytical experiments, every research chemist, whether he uses a "test tube" or a "пробирка," must calculate his findings in the international language of figures in two forms (weights and equivalents), and often three (weights, equivalents and percent-equivalents), and then compare experimental data with theoretical values.

Technicians and statisticians working with the results of hydrochemical analyses performed at different times by different laboratories inevitably face the problem of converting their figures to one system.

All such calculations are considerably simplified by the use of the tables and nomograms in this book, originally published by the State Scientific and Technical Press for Literature on Geology and the Conservation of Mineral Resources, Moscow.

All the tables and nomograms are based on analytical results expressed in the form widely used in hydrogeological practice—milligrams per liter (weight form) and milligram-equivalents per liter (equivalent form). For calculation of percent-equivalents, the sum of cation equivalents and the sum of anion equivalents are taken as 100% each. Several new tables are presented for the first time, and the many tables for converting water-analysis results from one form to another make it possible to find the milligram-equivalents for any practically possible content of a component in water, accurate to the second decimal place, and the weight content of substances to tenths of a milligram.

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BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

A translation of BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY, a publication of the Academy of Medical Sciences of the USSR

Russian Original Dated November, 1960

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The National Institutes of Health of the Public Health Service has supported in part the cost of publishing BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE through the purchase of copies for distribution to American medical libraries.

LOCAL RESPONSE OF THE HUMAN ELECTROENCEPHALOGRAM TO LIGHT

L. M. Puchinskaya

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 3-8, November, 1960
Original article submitted September 23, 1959

In the human electroencephalogram (EEG), afferent stimulation causes either a depression or an increase in the original activity. In addition to the general changes, there are also local responses to stimulation, which are referred to as evoked responses. It has frequently been shown [4, 6, 8, 12] that, in man, local responses to light, sound, and proprioceptive stimulation may be recorded through the skull from the corresponding projection areas. They evidently correspond to the "primary" responses found in animals, though they do not appear to be the same, on account of the method by which they are recorded. For this reason it is better to refer to them as the "local specific response."

The responses are not easily recorded through the skull because their voltage is small compared with the spontaneous EEG potentials. In order to obtain a specific response to light in the human EEG, most authors have disposed the electrodes sagittally and parasagitally over the occipital protuberance, using bipolar vertical leads [6, 12].

It has frequently been shown [1, 3, 7, 9] that application of stimuli to different sensory modalities in addition to producing the specific response causes also a response which is best shown in the vertex region and which may take the form of either a rapid oscillation, a slow wave, or a series of oscillations (vertex complex). Many authors have associated this nonspecific local response with the activity of the thalamic reticular formation. Both the amplitude and the latent period of the nonspecific response are greater than those of the specific response.

The present investigation represents an attempt to determine the conditions under which a well-marked specific response to light may occur in the human EEG, as recorded on an ink-writing electroencephalogram.

A comparison was made of the specific and the nonspecific responses to light, because in many cases when extraneous stimuli have been used the nonspecific effect has been much better shown and more widely distributed than the specific.

METHOD

The observations were carried out on 21 healthy human subjects between 18 and 36 years old and on three older individuals; in all, 35 records were made. In many cases, other studies were made of patients with local brain damage, the EEG being recorded on an ink-writing electroencephalogram. Electrodes made by the firm Al'var were used. Light flashes were supplied by a Kaiser photostimulator, or by a Soviet FFS-1 instrument. The subjects were seated and their eyes were open; the stimulus lamp was placed level with the eyes at 50-70 cm distance.

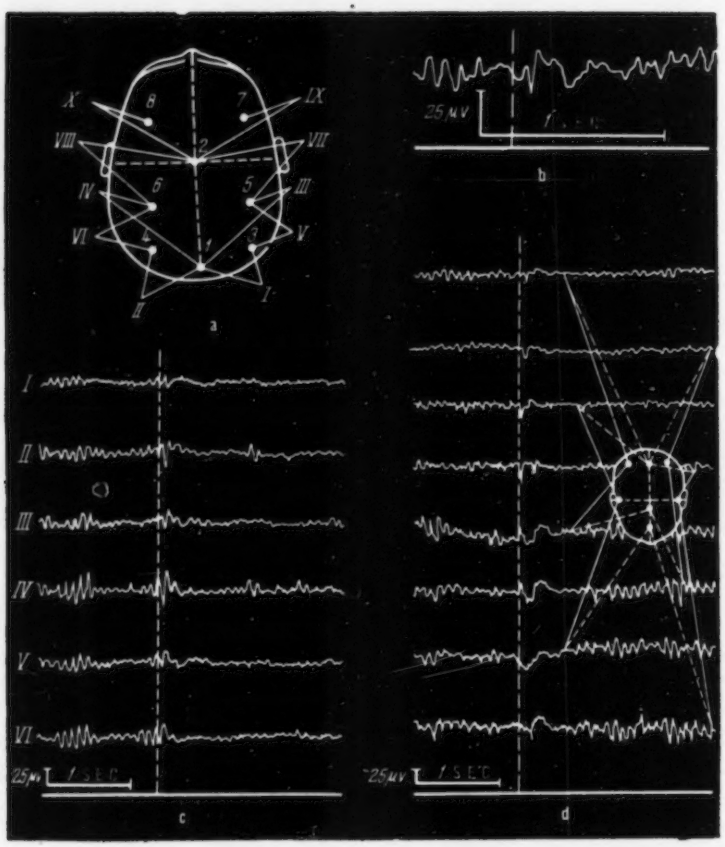

Fig. 1. Arrangement for obtaining the nonspecific response to light in the human electroencephalogram. a) Diagram of the leads. Disposition of the electrodes: sagittal electrodes: 1) over the occipital protuberance; 2) over the vertex; 3, 4) occipital leads; 5, 6) posterior parental electrodes; 7, 8) posterior frontal electrodes. b) A specific response to a single flash of light; c) well-shown specific effect to light in different leads from the occipital region; d) distribution of the specific and nonspecific responses to light according to areas.

The position of the electrodes is shown in Figs. 1, 2, and 3.

For the specific response to light leads I-II, III-IV, and V-VI were used, and for the nonspecific response leads VII-VIII and IX-X. In many of the investigations, there were very small deviations from the disposition of the electrodes illustrated.

RESULTS

The response to a single flash of light takes the form of rapid oscillation followed by alpha-like waves (Fig. 1b). It is best shown in the leads I-II, III-IV (Fig. 1c). As can be seen from the curves of the vertex leads V-VI, it is absent or less well developed in the first two leads. However, it is just this arrangement of leads which is most often used by other investigators. Sometimes the specific response to light spreads to the bordering anterior areas, but it was always best shown in the sagittal lead I, and the effect is evidently due to the disposition of the visual fields on the medial surface of the hemispheres.

When there is also a nonspecific response to light its distribution according to areas is clearly seen (Fig. 1d). In the curve given, the specific response to light takes the form of a rapid oscillation, while the nonspecific effect

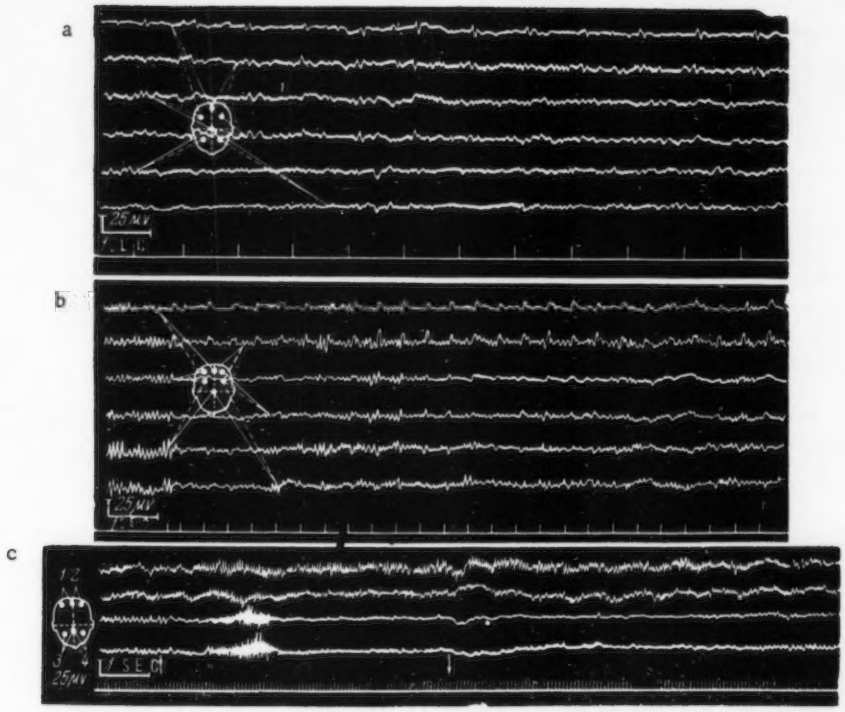

Fig. 2. Specific and nonspecific responses in the human electroencephalogram. a) Extinction of the nonspecific response to light by rhythmical stimulation, with preservation of the specific effect; b) increase of the specific response to light during stimulation; c) increase in the specific response to light during clenching fist in a patient with a tumor of the frontal bone.

is represented by a slow biphasic wave. In leads I-IV a specific effect is shown, and in leads V-VI there is also a specific effect followed by a nonspecific response. In leads VII-VIII, there is a nonspecific response only.

The specific and nonspecific responses differ not only in the latent period, shape, and in the region from which they are recorded, but they differ also when rhythmic stimuli are applied. It can be seen from the curves of Fig. 2a that at a flicker frequency of 1 per second, the nonspecific component disappears after 6 seconds. At a higher frequency the nonspecific effect develops only in response to the first flash. However, the specific effect to rhythmic light stimulation does not die out. Further, as the stimulation is continued, the specific response to light increases in amplitude and becomes more regular (Fig. 2b).

An increase in the not very clearly shown response to light can also be observed when other afferent stimuli are produced by sound, or by tightly squeezing the fist. In some cases of local cerebral damage, this increase was very marked. For instance, Fig. 2c shows the EEG of a patient with a tumor of the frontal bone which extended intracranially and compressed the frontal lobe. When rhythmic light stimuli were applied, there was a specific response to light in leads I-II, which followed the stimulus rhythm. In leads III-IV the Rolandic rhythm was recorded, and it did not change when light stimuli were applied. When the patient closed his fist more tightly, the Rolandic rhythm was depressed, and there was an increase in the light response in leads I-II. When the fingers of the fist were relaxed, the Rolandic rhythm gradually recovered, and at the same time the response to light became less marked.

In many cases, when the specific response to light was well shown, it became reduced when the afferent stimulation was continued.

A factor which increases the specific response to light is a general illumination during the investigation.

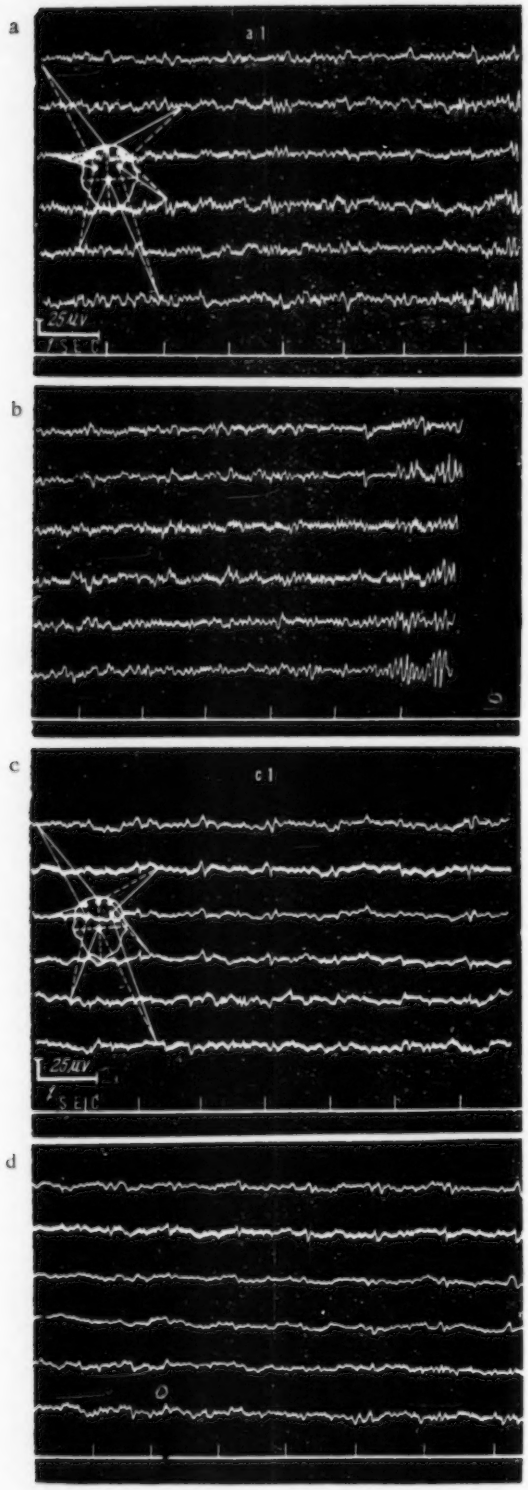

Fig. 3. The specific response to light in darkness and in general illumination in the same subject. Key: a1) In the dark; c1) with a light background.

Figure 3 shows EEGs recorded from the same subject in the dark, and in general illumination. The response to rhythmic light stimulation at a frequency of 1-2 per second in darkness is poorly shown both at the beginning (Fig. 3a) and at the end (Fig. 3b) of the investigation. The response to stimulation at the same frequency obtained in general illumination is well shown at the beginning (Fig. 3c), and at the end (Fig. 3d). With general illumination the specific response to light can as a rule be recorded over a wider region, and in particular in leads VII-VIII.

The results obtained on the increase in the amplitude of the specific response to light when additional afferent stimulation of the auditory or proprioceptive receptors is given during general illumination in man agrees with the results obtained in animal experiments.

Chang [5] showed that the electrical response of the visual cortex to stimulation of the lateral geniculate body and the response of the auditory cortex to stimulation of the medial geniculate body are increased in amplitude by illumination of the retina; the threshold of the responses is also reduced. However, a very bright light may suppress the responses. Gellhorn [10] found that in the cat under dial anesthesia, the primary responses of the visual and auditory areas were increased in amplitude during painful stimulation, and became more regular and extended over a greater area of the cortex. Other results have been obtained which are the reverse of those described above. Thus, in animal experiments, S. P. Narikashvili [2] found that when the preparation was in good condition and when the strength of the stimuli was appropriately chosen, sound stimulation might exert a depressive effect on the primary response of the visual area, and light stimulation might similarly depress the response of the auditory area.

Studies on man have been made by Jasper and Cruishank [11]. They studied the response of the human EEG to light, and found that the response was maximal in amplitude and most regular in cases when the muscles were relaxed and the eyes were closed.

The reason for these contradictions was evidently due to the different conditions under which the experiments were carried out and the different kinds of strength of stimuli used.

All the facts we have considered show that a current of nervous impulses passing along one afferent pathway will affect the activity of another system, will reflect the excitability of the brain and in so doing will establish a background on which the activity

of any other afferent system will be manifested. Evidently the change in excitability concerns not only the cortical neurons, but the whole afferent system. Chang [5] has shown that when the whole of the grey matter of the brain is removed, the effect of the increase of the primary response to illumination of the retina is preserved. Probably such effects are mediated through the nonspecific projection system.

SUMMARY

A study was made of the local specific response to light in the human EEG, registered by bipolar recording with a sagittal occipital electrode. It was found that background illumination combined with additional sound or proprioceptive stimulation caused the amplitude of the response to increase, to become more regular, and to spread to adjacent areas.

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A PHYSIOLOGICAL STUDY OF THE TRANSVERSE BALLISTOCARDIOGRAM

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 8-13, November, 1960
Original article submitted October 18, 1959

The ballistocardiogram was developed in 1951 as a method of studying cardiac activity [1], and in recent years there have been extensive theoretical and clinical applications. It is a method of recording the kinetic processes associated with cardiac activity and with the movement of the blood in the main vessels. The receptive element of the apparatus produces an analysis of the forces acting on it, these forces originate in the thoracic cavity of the subject, who lies on a special table. The principal record is that of the projection of the center of gravity of the thoracic cavity on to the receptive plane of the instrument.

It has been shown [2] that a sufficiently complete account of the movement of the center of gravity of the human thoracic cavity may be obtained by means of two leads of the ballistocardiogram, the longitudinal and the transverse. The first of these (BCG-1) records the displacement along the longitudinal body axis. Displacements perpendicular to the longitudinal axis are recorded by the transverse lead, BCG-2.

We here report a study of the changes in the transverse lead.

The longitudinal ballistocardiogram has by now been adequately studied, but little work has been done on the transverse component. The BCG-2 component has been used as an additional recording in vector ballistocardiography [2, 3, 4], and it has also been studied in connection with the work of the heart in patients with hereditary heart disease [6]. In this connection, a physiological analysis of the transverse ballistocardiogram is not only interesting as a method, but it also has practical applications because, as will be shown below, it enables the duration of ventricular systole to be determined clinically in diagnosing various cardiac diseases.

METHOD

The transverse ballistocardiogram is recorded by means of a two-channel addition to a "Biofizpribor" electrocardiograph. The pick-up device responds to the displacement of the center of gravity of the thorax both longitudinally and transversely. For transverse recording, the device is connected so that a displacement of the load from the patient's right to left gives a negative deflection of the oscillograph beam.

Simultaneously, a recording is made of: the electrocardiogram (lead II), heart sounds, longitudinal ballistocardiogram, and the sphygmogram of the carotid artery. All the curves are on the same time scale. The rate of movement of the photographic paper is either 50 or 100 mm/sec. The time marker consists of vertical lines extending across the whole 120 mm width of the paper at 0.02 sec time intervals.

Because the pulse wave reached the carotid artery at an appreciable time after the moment of opening of the semilunar valves, when comparing this curve with the transverse ballistocardiogram we have introduced a time correction for the rate of spread of the pulse wave. For each separate curve this correction was calculated, by Schulz's method.[13], as the time interval between the second sound and the notch on the carotid pulse curve. made on 70 healthy human subjects between 20 and 40 years old.

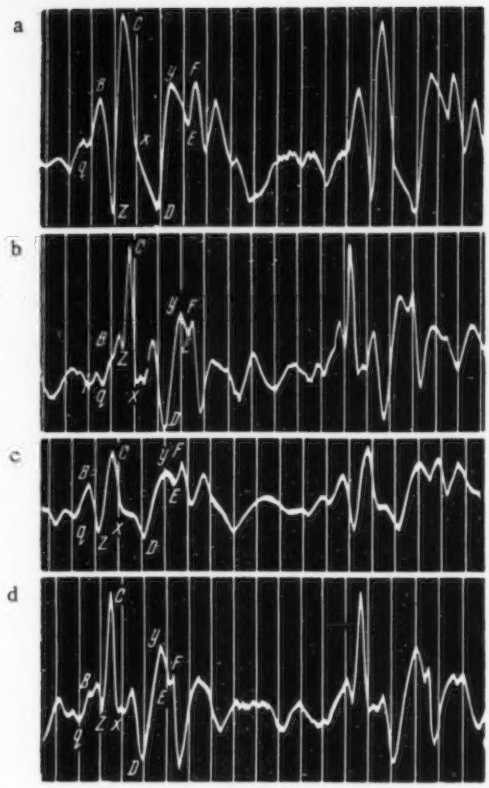

Fig. 1. Variants of the normal transverse ballistocardiogram. The interval q – B corresponds to phase of asynchronous contraction, the interval B – z represents the phase of isometric contraction, the z – y interval corresponds to the expulsion of blood from the ventricles, and the y – E interval represents the protodiastolic period.

In addition to making a physiological analysis of the transverse ballistocardiogram, we also studied the shape of the recording itself.

We here report the results of investigations made on 70 healthy human subjects between 20 and 40 years old.

RESULTS

The transverse ballistocardiogram consists of a curve which repeats at each heart beat, and whose shape shows the same characteristic features in different investigations. The principal waves of the systolic complex have been described by us so as to correspond to those of the longitudinal ballistocardiogram, and are designated q, B, z, C, D, E, and F.

The individual features concern essentially the depth of the z wave and the height of the C wave. In some cases the B wave is toothed (Fig. 1).

As is shown in Fig. 2a, all the waves of the BCG-1 and BCG-2 are strictly synchronous (except for the D wave). At the same time, on the transverse ballistocardiogram there are always two waves (the x and y waves) for which in some cases there is no corresponding wave on the BCG-1. The values for the duration of the BCG-2 waves are given in Table 1. Individual variations in the duration of the intervals indicate features of the cardiac contraction, and are also associated with variations in the cardiac rhythm in the different studies.

In 80% of the cases a comparison of the transverse ballistocardiogram and the electrocardiogram showed that the break in the curve q (Fig. 2b) corresponds to the beginning of electrical systole (Q wave). In other cases, the onset of depolarization of the ventricles is not indicated on the BCG-2, evidently because of the small mechanical effect of the asynchronous contraction.

When the transverse ballistocardiogram is compared with the phonocardiogram, it is seen that the beginning of the high frequency oscillations of the first sound corresponds precisely to the point B of the transverse ballistocardiogram, and the onset of the second sound occurred at the same time as the point E (see Fig. 2b).

In the carotid pulse curve produced as described above, the beginning of the anacrotic notch corresponds to the apex z on the BCG-2, and the lowest point of the notch on the pulse curve corresponds to the point E on the BCG-2. In all cases the point y on the BCG-2 corresponds clearly with the point at which the catacrotism changes into the notch (Fig. 2b).

The relationship between the transverse ballistocardiogram and the electrocardiogram, phonocardiogram, carotid pulse curve, and longitudinal ballistocardiogram is summarized in Fig. 3.

The comparison enables a physiological evaluation of some of the waves of the systolic complex of the transverse ballistocardiogram to be made. The first of these, the q wave, evidently represents the phase of what Holldack has called the phase of change of shape of the ventricles, or, as we call it, the phase of asynchronous contraction. This is the time of occurrence of the successive contraction of the different parts of the ventricular myocardium, which causes them to change shape without increasing the intraventricular pressure.

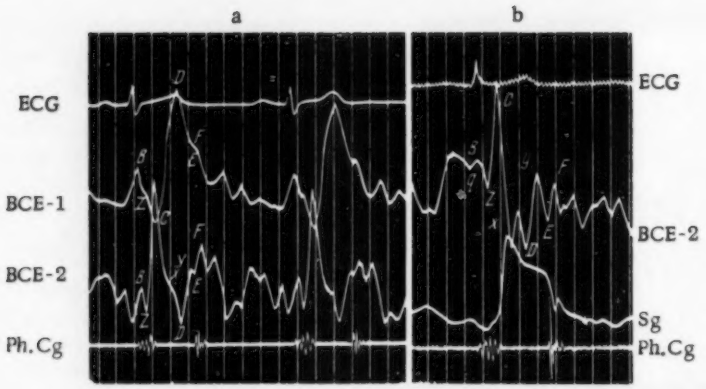

Fig. 2. Simultaneous records of the transverse ballistocardiogram (BCG-2) and the electrocardiogram (ECG), phonocardiogram (FCG), sphygmogram of the carotid artery (SG), and the longitudinal ballistocardiogram (BCG-1). Description in text.

TABLE 1

Values of the Time Intervals of the Transverse Ballistocardiogram

Interval	Limits over which duration varies (in seconds)
q – B	0.04 – 0.07
B – z	0.02 – 0.05
z – C	0.03 – 0.06
C – x	0.03 – 0.06
x – D	0.08 – 0.12
D – y	0.06 – 0.10
y – E	0.02 – 0.05
E – F	0.01 – 0.04

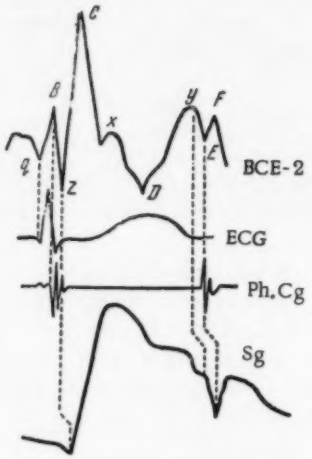

Fig. 3. Diagram comparing the transverse ballistocardiogram with curves recorded by other methods (indications as in Fig. 2).

The B wave of the transverse ballistocardiogram occurs at the moment of closure of the atrioventricular valves, as is shown by the fact that it coincides with the onset of the high frequency oscillations of the first sound. Holldack [11] and others after him [8, 12] have used this phonocardiographic criterion to determine the onset of the phase of increased intraventricular pressure or the phase of isometric contraction. Consequently, the point B of the transverse ballistocardiogram represents the commencement of mechanical systole of the ventricles.

The accurate correspondence of the point z of the transverse ballistocardiogram with the beginning of the anacrotic notch on the sphygmogram of the carotid artery indicates that the point z represents the beginning of the phase of expulsion of blood from the ventricules, or, what is the same thing, the end of the phase of isometric contraction.

Further comparison of the ballistocardiogram and carotid pulse curve enables the physiological significance of the y and E waves to be elucidated. The correspondence of the former with the beginning of the notch, and of the latter with the lowest part on the curve indicates that the point y on the transverse ballistocardiogram represents the beginning, and the point E the end of the protodiastolic period.

The protodiastolic period as defined by Wiggers [14] follows after the phase of expulsion of the blood from

TABLE 2

Mean Duration of the Phases of Cardiac Contraction in Healthy Subjects (in seconds)

Author	Phase of asynchronous contraction	Phase of isometric cohtraction	Expulsion phase	Mechanical systole	Proto-diastole	Method of investigation
Holldack [11]	0.051	0.037	0.290*	0.327	–	Polycardiography
Nassi and others [12] . . .	0.050	0.032	0.289*	0.321	–	The same
E. B. Babskii and V. L. Karpman [5] . . .	0.053	0.030	0.284*	0.314	–	" "
E. B. Babskii and V. L. Karpman [5] . . .	0.052	0.029	0.287*	0.316	–	BCG-1
Our own results	0.053	0.032	0.280	0.312	0.035	BCG-2

*The duration of the protodiastolic period is included in the expulsion phase.

the ventricles and represents the time expended in closing the semilunar valves. The onset of the protodiastolic period (end of the expulsion phase) is determined from the beginning of the notch on the pressure curve of the great vessels [14]. Broemser [9] has shown that this moment may be determined also from the carotid pulse curve, the beginning of the notch on it corresponding to the end of the phase of expulsion of the blood from the ventricles. However, the end of the protodiastolic period (the time at which the closure of the semilunar valves is completed) corresponds to the lowest point of the notch on the carotid pulse curve and to the beginning of the second heart sound.

The results obtained show that many of the waves of the transverse ballistocardiogram are associated with mechanical processes which represent the beginning or end of the different phases of contraction of the ventricles. It is clear that the duration of the different systolic phases may be determined from the interval between the relevant waves on the BCG-2. Thus, the q – B interval represents the duration of the phase of asynchronous contraction (the time from the beginning of electrical depolarization of the ventricles to the time of closure of the auriculoventricular valves). The B – z interval corresponds to the phase of isometric contraction (the period during which the valves are closed). The duration of both intervals corresponds to Blumberger's phase of tension [7].

The next, systolic, or expulsion phase, was previously considered as the part of the cardiac cycle during which the semilunar valves were open. However, after the work of Wiggers [14] it became clear that actually the time during which blood is expelled from the ventricles is somewhat shorter, because the closure of the semilunar valves takes place during the relaxation of the myocardium, and therefore cannot be included in the expulsion phase. This fact is revealed in many works on the analysis of the phases of cardiac contraction [10, 15]. Nevertheless certain authors [7, 11, 12] continue to include the protodiastolic period in the expulsion phase. Evidently, in that case, the duration of this phase will be relatively increased.

As has been pointed out previously, in the transverse ballistocardiogram it is possible to distinguish the protodiastolic period (y – E interval) and, consequently, to determine the duration of the actual expulsion phase (interval z – y).

Because the duration of mechanical systole includes the times of isometric and of isotonic contractions, it may be determined from the B – y interval of the transverse ballistocardiogram.

Table 2 shows the durations of the different phases of ventricular systole as revealed by the transverse ballistocardiogram in the healthy human subjects whom we studied, as well as the results previously published by other authors who used different methods.

It can be seen from Table 2 that the phasic analysis of cardiac contraction from the ballistocardiogram is quite precise, so that it can be used for physiological and clinical investigations.

Finally we may note that the analysis of the phases of cardiac contraction which we have made by means of the ballistocardiogram has some advantages when compared with the corresponding information revealed by

longitudinal ballistocardiography, as some of the waves are more clearly shown. Also, no reliable determination has been made of the significance of the point y on the BCG-1. However, the B wave on the BCG-1 is often more clearly shown than on the BCG-2. For an analysis of the different phases, it is therefore advantageous to use both the ballistocardiograms.

SUMMARY

For the first time a physiological analysis has been made of the transverse ballistocardiogram. The curve was found to indicate the different phases of the cardiac cycle. It was shown that in man under normal conditions the phase of asynchronous contraction lasts 0.053 seconds, the phase of isometric contraction for 0.032 seconds, the ejection phase for 0.280 seconds, mechanical for 0.312 seconds, and the protodiastolic period for 0.035 seconds. An accurate analysis of the different phases of the different cardiac contractions by means of ballistocardiography can be made by recording the longitudinal and transverse ballistocardiograms simultaneously. The transverse ballistocardiogram shows a considerable degree of consistency in different healthy individuals.

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THE MECHANISM OF CORTICAL CONTROL OVER THE THYROID

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(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp 13-18, November, 1960
Original article submitted June 25, 1959

We have shown previously [1] that in conditioned reflex defensive activity, cortical influences reach the thyroid by the humoral path.

We here report a study of the problem of whether the humoral connection between the cortex and the thyroid is universal, or whether the transmission may change according to the influence of the surrounding medium on the central nervous system.

METHOD

The experiments were carried out on nine dogs. The functional condition of the central nervous system was altered by means of feeding conditioned reflexes. Conditioned reflexes were established in response to the sound of a metronome at 120 beats per second, and to intermittent light from a 50 w electric lamp. Inhibitory conditioned reflexes were developed to a metronome operating at 30 beats per minute, and by intermittently extinguishing positive conditioned reflexes. The activity of the thyroid gland was estimated from its content of radio iodine. Radio iodine was given orally, as was the sodium salt, the dose being 0.7 μC per kg. The experiments were carried out 48 hours or more after injecting I^{131}. The radioactivity of the thyroid was determined immediately after presenting the conditioned stimuli, after 15 minutes, subsequently at 30 minute intervals for 3-4 hours. The change was expressed as a percentage of the original value of the radioactivity which was assumed to be 100%.

RESULTS

At the outset, a careful study was made of the variation of the radio iodine content of the thyroid while the dogs were kept in the room and in the conditioned-reflex test compartment. It was found that in neither place did the level vary by more than ±10%, a result which showed that the conditions of the experiment themselves did not cause appreciable changes in thyroid activity. In this way we determined the normal level during the period of the experiments. The animal was then given a meat powder while it was in the conditioned-reflex test compartment, the portions consisting of 25 g which were given at 5 minute intervals 5-6 times during one experiment. It was found that here too the thyroid gland activity did not vary by more than ±10%. It was therefore, established that the food stimulus is without effect on thyroid activity.

The next stage was to determine the effect of the conditioned food stimuli on the thyroid activity.

Even the first experiments in elaborating feeding conditioned reflexes showed that a considerable change in the level of thyroid radio iodine occurred.

It can be seen from Fig. 1 that when conditioned reflexes are developed to M_{120}, the amount of radio iodine in the thyroid gland is reduced and that the low level is maintained for 2-3 hours, after which it returns to its

TABLE 1

Effect of the Development of Conditioned Feeding Reflexes on the I^{131} Content of the Thyroid in Hypophysectomized Animals

Date of experiment	Original radioactivity (in imp/min)	Change in the I^{131} content during time:									
		30 min		1 hr 5 min–1hr 20 min		2 hr 5 min – 2 hr 20 min		3 hr 5 min–3 hr 20 min		4 hr 5 min–4 hr 20 min	
		imp/min	% change	imp/min	% change	imp/min	% change	imp/min	% change	imp/min	% change
1958 г.											
1/VII	1 184	1 180	—0,3	1 120	—5,4	920	—22,3	928	—21,6	992	—16,2
3/VII	799	799	0	863	+8,0	799	0	799	0	863	+ 8,2
12/VII	384	336	—12,5	272	—29,2	224	—41,7	240	—37,5	341	—11,2
14/VII	288	224	—22,3	176	—38,9	208	—27,8	240	—16,7	320	+11,1
21/VII	153	99	—35,4	71	—53,7	82	—46,5	102	—33,4	108	—29,5
1959 г.											
26/I	2 832	2 152	—24,1	1 824	—35,6	1 872	—33,9	1 936	—31,7	1 936	—31,7
27/I	2 000	1 744	—12,8	1 744	—12,8	1 808	— 9,6	1 872	— 6,4	1 936	— 3,2
30/I	1 376	1 108	—19,5	1 044	—24,2	1 140	—17,2	1 220	—11,4	—	—
2/II	1 040	770	—26,0	720	—30,8	752	—27,7	810	—22,2	878	—15,6
3/II	1 040	844	—18,9	878	—15,6	944	— 9,3	966	— 7,2	990	— 4,8

original value. With training, the response of the thyroid to the conditioned stimulus is reduced, and disappears when well reinforced conditioned reflexes are established (See Fig. 1). The development and reinforcement in a single animal of a conditioned reflex to a second new stimulus, for example, to intermittent light, produces a change in thyroid gland activity similar to that which has just been described.

The effect of inhibition (extinction) on the secretion of the labelled hormone is increased in some animals and in others it is depressed (see Fig. 1). M. A. Usievich [2] observed a rather similar effect when studying the influence of the cerebral cortex on visceral function. He was able to show that the greatest change in the activity of the stomach, pancreas, liver, and other organs occurred during the period when either positive or inhibitory conditioned reflexes were being elaborated.

I. N. Knipst, T. A. Korol'kov, and M. N. Livanov [3] also showed that the synchronization of cortical activity was best shown during the development of a conditioned reflex and was maintained for a short while after it had been elaborated, i.e., when it was most completely generalized. The increased synchronization of cortical electrical activity was also observed when the conditioned reflexes were being extinguished.

The results reported show that in contrast to unconditioned reflexes, for conditioned reflexes the whole set-up of the experiment and the application of conditioned stimuli to dogs is a very delicate task. Conditioned reflex activity requires a considerable potential in the cerebral cortex, particularly when the animal is first confronted with a new stimulus which induces widespread excitation of the central nervous system. This result is reflected in some way in the operation of the viscera, and, as our experiments have shown, on the work of the thyroid. As conditioned reflex training continues, the excitation becomes concentrated, and a well-developed conditioned reflex has no appreciable effect on visceral function, or, in particular, on the operation of the thyroid.

Thus, the results obtained for the thyroid indicate the immensely important part played by the functional condition of the central nervous system, or, more accurately stated, by the condition of the cells of the cerebral cortex in controlling endocrine activity.

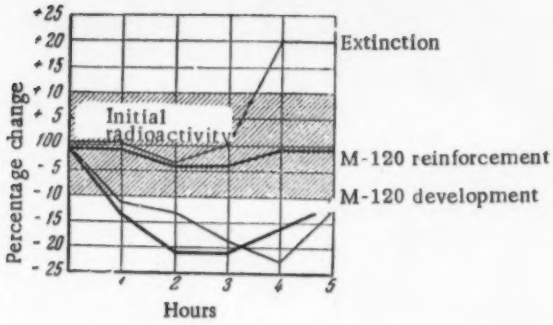

Fig. 1. The effect of conditioned food stimuli on the I^{131} thyroid gland content.

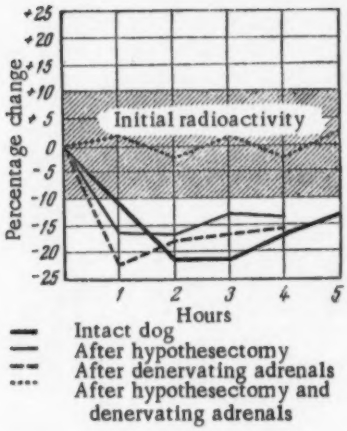

Fig. 2. Change in the amount of I^{131} in the thyroid gland of a dog during feeding conditioned reflex activity. Experiment conducted after breaking the connection between the central nervous system and the adrenals.

It was extremely important to determine in what way the influence of the central nervous system on the thyroid is conveyed. Using the method described previously [1], we have studied the transmission path from cortex to thyroid in three sets of experiments. It was first required to determine whether the cortical influence travels along the same pathway as it does during defensive conditioned reflex activity.

The first experiments were carried out on hypophysectomized animals in order to determine whether the hypophysis is an essential link in the transmission between cortex and thyroid. The animals were used for the experiment three weeks or more after removing the anterior lobe of the hypophysis. In the operated animals, it was difficult to develop conditioned reflexes; thus, in one operated dog, the first conditioned reflex appeared on the 17th combined application of the conditioned and unconditioned stimuli. As a rule, the latent period varied between 3 and 5 seconds, and sometimes was as long as 7 seconds. The reflexes were difficult to reinforce, and frequently even after reinforcement, it was difficult to obtain a prompt response to the initial stimulus. Hypophysectomy therefore produced a definitely deleterious influence on higher nervous function.

As can be seen from Table 1, the effect of the formation of conditioned feeding reflexes in the hypophysectomized animals was to increase the secretion of radio iodine from the thyroid. In most cases this response appears immediately at the end of stimulation; during the next 2-3 hours the reaction increases further, and then gradually declines. The same result was obtained in almost all the experiments of this series. The results shown in Table 1 indicate that the cortical effect on thyroid gland function can take place without the involvement of the hypophysis, a conclusion which is in line with the results which we obtained on defensive conditioned reflex activity.

In the next set of experiments we studied the part played by the adrenal glands in the transmission of the cortical influence on thyroid function. The animals were used for the experiment after removing the anterior pituitary lobe, and after separating the adrenals from the central nervous system by dividing the left-splanchnic nerve and removing the right adrenal gland. In these animals, conditioned reflex activity was approximately the same as in the hypophysectomized dogs.

It can be seen from Table 2 that food conditioned stimuli had no effect on thyroid function. Variations in the level of radio iodine in the gland did not exceed ±10 %. As was pointed out previously, such variations are within normal limits. Consequently, no change in thyroid function is caused by the elaboration of conditioned reflexes. Therefore, in this case cortical influence does not extend to the thyroid gland. In these animals, under the influence of internal inhibition the amount of radio iodine in the thyroid gland again does not exceed the normal limits of variation. Thus, after removal of the hypophysis and destroying the connection between the

TABLE 2

The Effect of the Development of Positive and Inhibitory Feeding Conditioned Reflexes on the I^{131} Thyroid Gland Content after Hypophysectomy and the Disassociation of the Adrenals from the Central Nerous System

Date of experiment	Initial radio-activity (in imp/min)	Change in the I^{131} content during time: 30 min		1 hr 5 min - 1 hr 20 min		2 hrs 5 min. - 2 hrs 20 min		3 hrs 5 min - 3 hrs 20 min		4 hrs 5 min.- 4 hrs 10 min.		Remarks
		imp/ /min	% change	imp/ /min	% change	imp/ /min	% change	imp/ /min	% change	imp/ /min	% change	
1958 r.												
1/IV	440	440	0	440	0	440	0	440	0	440	0	
3/IV	362	362	0	362	0	362	0	362	0	362	0	
5/IV	238	238	0	238	0	238	0	238	0	238	0	
7/IV	240	240	0	240	0	240	0	240	0	240	0	
24/IV	17 114	18 394	+7,4	16 538	−3,4	19 226	+12,3	16 538	−3,4	—	—	
28/IV	13 904	13 200	−4,9	13 392	+3,8	13 712	−1,3	13 520	−2,8	14 032	+0,9	
29/IV	12 880	12 304	−4,6	12 816	−0,5	12 672	−1,7	12 816	−0,5	12 176	−5,5	
4/V	7 834	7 898	+1,0	8 154	+4,0	8 090	+3,2	8 026	+2,4	7 962	+1,6	After injecting thyrotropic hormone
7/V	6 409	6 537	+1,9	6 281	−2,0	6 153	−4,0	6 089	−5,0	6 163	−4,0	
8/V	5 836	6 156	+5,4	5 644	−3,7	5 900	+1,0	5 836	0	5 836	0	
16/V	3 982	4 110	+3,2	4 110	+3,2	4 174	+4,9	4 110	+3,2	4 046	+1,6	
22/V	1 557	1 493	−4,1	1 557	0	1 429	−8,3	1 493	−4,1	1 493	−4,1	
23/V	1 316	1 380	+4,8	1 380	+4,8	1 380	+4,8	1 380	+4,8	1 380	+4,8	Extinction
24/V	1 120	1 184	+5,7	1 184	+5,7	1 120	0	1 120	0	1 184	+5,7	»
26/V	989	989	0	989	0	989	0	989	0	989	0	»
8/XII	943	922	−2,3	937	−0,7	928	−1,6	928	−1,6	928	−1,6	
10/XII	825	883	+7,0	843	+2,2	873	+5,8	857	+3,9	873	+5,8	
12/XII	666	650	−2,5	666	0	666	0	660	0	666	0	Differentiation
13/XII	374	366	−2,2	382	+2,1	382	+2,1	382	+2,1	—	—	
15/XII	407	444	+9,0	407	0	453	+11,3	425	+4,4	417	+2,4	

adrenals and the central nervous system, neither the development of positive conditioned reflexes nor the establishment of internal inhibition exerts any influence on the secretion of labelled thyroid hormone.

Because it is known that thyroid function is reduced by hypophysectomy, it would be expected that the failure of the thyroid to respond to cortical impulses would occur as a result of its reduced level of activity. In order to discount this explanation, it was required to stimulate thyroid function in these animals, which was done by repeatedly injecting thyrotropic hormone. Thus, in the dog Lorta, 7 months after operation the maximal absorption of radio iodine was reduced from the normal value of 22.4% to 4.8%. After subcutaneous injections of 0.5-1 ml of the thyrotropic hormone "Ambinon", the maximum absorption of radio iodine by the thyroid had increased to 45%. The results of experiments carried out on animals in whom the thyroid gland had been stimulated in this way were no different from those described above (see Table 2).

The results of this set of experiments completely agree with those obtained in the study of defensive conditioned reflexes. After hypophysectomy and the interruption of the nerve supply to the adrenals, all possibility of the transmission of cortical influence to the thyroid gland had been excluded.

The problem then arose as to whether the adrenals are normally involved in the transmission of cortical influence to the thyroid, when the hypophysis is still present.

After severing the nerve supply from the central nervous system to the adrenals as described above, the development of conditioned relfexes was associated with a greater excretion of labelled hormone into the blood stream (Fig. 2). This result shows that in this case the link between the central nervous system and the thyroid includes the hypophysis. For the sake of clarity, in Fig. 2 the results for all three sets of experiments are shown.

The following conclusions may be made from the results obtained: the effect of the cerebral cortex on the liberation of thyroid hormone can occur only when either the hypophysial or the adrenal hormonal link is present.

Because the existence of this hormonal link is essential for the transmission of both defensive and feeding conditioned reflexes, i.e., for the realization of different influence from the surrounding medium, it may be taken as established that humoral transmission is a quite general means of association between the central nervous system and the thyroid gland.

SUMMARY

The action of the cerebral cortex upon the thyroid gland is effected humorally during both feeding and defensive reflex activity.

In both instances either the hypophysis or the adrenal glands are involved. It is concluded that humoral transmission is quite a general form of connection between the cerebral cortex and the thyroid.

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*Original Russian pagination. See C. B. translation.

THE PART PLAYED BY THE SKELETAL MUSCLES IN THE CHANGES IN GASEOUS EXCHANGE IN HYPER- AND HYPOTHERMIA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 19-23, November, 1960
Original article submitted December 23, 1959

Cooling human beings and animals causes the development of weak electrical activity in "resting" muscles, a condition which has been referred to as thermoregulatory tone. The effect was first discovered by Burton and Bronk [9], and the work was extended by other investigators [2, 3]. It is thought that the electrical activity of the muscles during their apparent resting condition indicates changes in muscular metabolism, and constitutes an important mechanism of chemical thermoregulation.

The present work describes a parallel study of gaseous exchange and electrical muscular activity at different body temperatures reached initially by the application of heat and then by the induction of deep hypothermia.

Variations in gaseous exchange at different body temperatures have been studied by several investigators [2]. In white rats, the corresponding changes in oxygen consumption have been studied in detail in our laboratory by A. Izbinskii [4] and A. I. Shcheglova [7]. Because the muscles play an important part in chemical thermoregulation, an attempt to relate changes in gaseous exchange to variations of the electrical activity in the muscles is extremely important. (After our work had been completed, a report was published by I. S. Repin [5] describing thermoregulatory tone in cooled rabbits.)

METHOD

The work was carried out on white rats weighing 180-200 g. The animals were placed in narrow cages having wire sides and top and a wooden floor. Such an arrangement limited their movements, and enabled any necessary manipulations to be carried out while a normal body position was maintained. Measurements were made of the electrical activity of the muscles of the thigh, and in some cases of those of the back, neck and head. The apparatus and method for recording the muscular potentials has been described in detail previously [2, 3]. Gaseous exchange was determined in pneumatically sealed chambers of 15 liters capacity. Air samples were analyzed by means of a Haldane apparatus. Body temperature was measured by a thermocouple inserted 2.5 cm into the rectum.

The rats were placed in a hermatically sealed chamber which was initially at 35°. When the temperature in the rectum reached 38.5-40°, the chamber was ventilated and the gaseous exchange and electrical muscular activity were determined. The animals were left in the room for 20-25 minutes. The next experiment was carried out in the same way at a temperature of 19-22°, the temperature in the rectum being 35-36°; the temperature of the chamber was then reduced to 8–10° when the rectal temperature fell to 29-32°.

For greater cooling, the rats were immersed for a few seconds in water, and then transferred to a refrigerator at –5°. When the temperature in the rectum had fallen to 26-17°, gaseous exchange and electrical muscular activity

The Changes in the Oxygen Consumption in White Rats at Different Body Temperatures

Number of animals	Body temperature				
	39—40°	35—36°	29—32°	19—22°	18—17°
	Oxygen consumption (in ml/g/hr for mean)				
1	—	1,81	2,96	1,20	0,88
2	—	1,80	3,27	1,73	0,73
3	0,73	2,10	—	—	0,52
4	1,35	2,61	—	—	0,65
5	1,40	2,03	2,90	1,04	—
6	1,06	2,22	4,11	1,11	0,47
7	0,71	2,15	3,09	1,45	0,70
8	0,70	1,34	3,75	1,85	0,17
9	0,69	2,74	3,69	—	0,75
10	0,92	2,00	2,62	1,21	0,33
Average	0,94	2,08	3,29	1,19	0,57

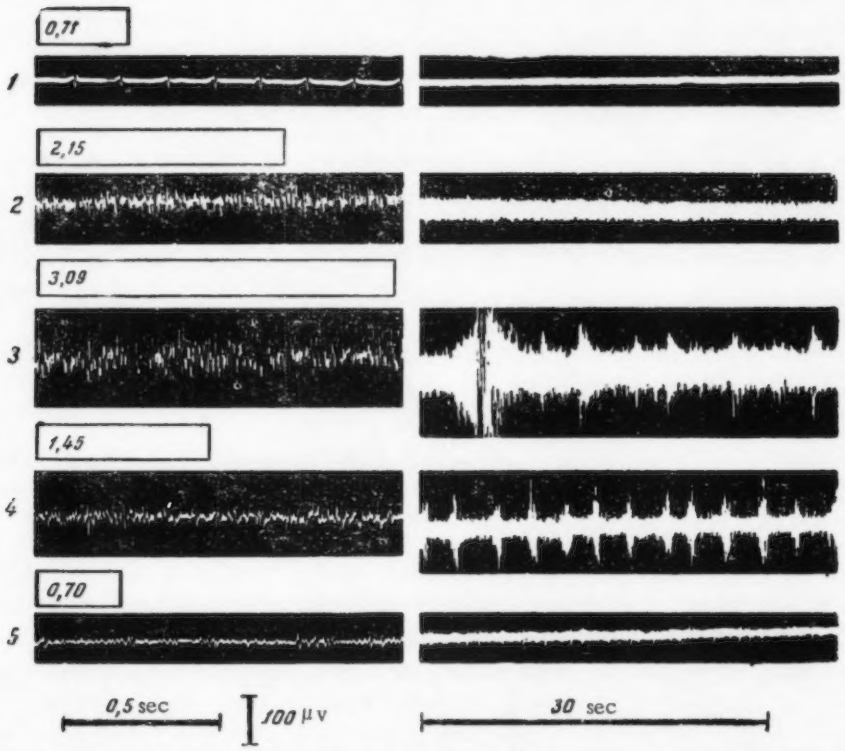

Fig. 1. Change in the oxygen demand and in muscular electrical activity in the thigh muscles of the rat at different body temperatures. 1) At a rectal temperature of 39.2° (the electrocardiogram is shown); 2) at 34.9° 3) at 31.4°; 4) at 21.5°; 5) at 18.5°. The electrocardiogram record was taken at a paper speed of 50 mm per second (on the left) and at a speed of 2 mm per second (on the right). The oxygen consumption (shown by the horizontal columns placed above the electrocardiogram) is given in ml per g per hour.

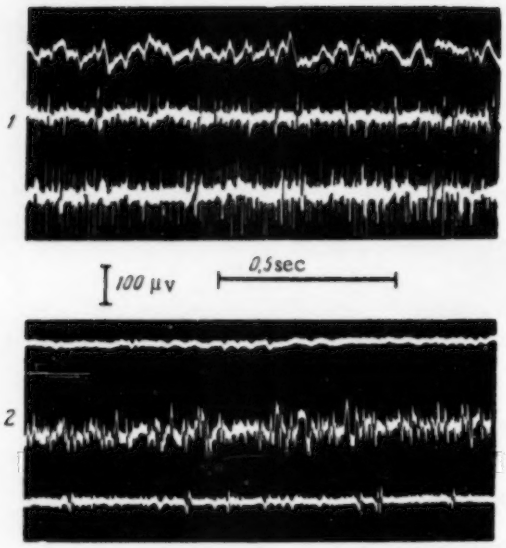

Fig. 2. Change in the electrical activity of the cerebral cortex and muscles in the hypothermic white rat. 1) Record taken for rectal temperature of 35.4°, temperature of temporalis muscle 34.4°, of the thigh muscle 34.1°; 2) after cooling to a rectal temperature of 11.5°, temperature of temporalis muscle 15.8°, thigh muscle 10°, and brain 15.9°. Curves from above downwards: potentials from cerebral cortex, from temporalis muscle, and from the thigh muscle.

were again measured. In this case the temperature of the gaseous-exchange chamber was 18-20°.

In other experiments, the electrical activities in muscles and in the cerebral cortex were recorded simultaneously. For this purpose the animals were fixed to a stand, and after the fur had been moistened with water they were surrounded by finely crushed ice. Platinum electrodes were introduced into the brain to a depth of 1-1.5 mm. Temperature measurements were made of the rectum, muscles, and brain. In all, 140 experiments were performed on 34 rats.

RESULTS

As can be seen from the table and from Fig. 1, for a body temperature of 38.5-40°, the minimum value of the gaseous exchange is attained when there is practically no electrical activity in the muscles. At a body temperature of 35-36°, the greater oxygen demand coincides with a comparatively feeble but maintained muscular electrical activity. This phenomenon of thermoregulatory tone is not associated with any movements, but may be observed during the whole of the experiment, while the animals remain motionless. The smallest observable movement of the rat causes a momentary burst of potentials of large amplitude (up to 300-600 μv or more).

At a rectal temperature of 29-32°, the oxygen consumption increases shaprly. Thermoregularoty tone increases to a maximum. In addition, shivering commences, and is indicated on the electromyogram by periodic bursts of potentials of large amplitude (up to 300-500 μv). Cooling the body until the rectal temperature falls to 22-19° causes a reduction in gaseous exchange and a depression of the electrical activity of the muscles. As can be seen from Fig. 1., there is then no appreciable change in the shivering, and at the same time the thermoregulatory tone is decreased. There is a reduction in the frequency and the voltage of the separate potential excursions. The peaks become rounded. Further cooling to a rectal temperature of 18-17° causes a marked reduction in gaseous exchange. The electrical activity of the distal parts of the body (hind legs and back) is depressed. However, some electrical activity is maintained in the muscles of the head and neck, and, as our subsequent experiments showed, there is even a depression of the cerebral potentials. We found that the potentials developed in the cerebral cortex disappeared at a brain temperature of 16-17°. The temperature in the rectum, in the muscles of the thigh, and in the temporalis muscle then were 11-15°, 10-14°, and 16-17°, respectively. In our experiments, the temperatures of the brain and of the temporalis muscle were always a few degrees higher than in the thigh and rectum (Fig. 2).

It was therefore found that the oxygen consumption and electrical activity of the main mass of skeletal muscles of the white rat were altered in the same direction over a range of temperatures extending from hyperthermia to profound hypothermia (while the animal remained apparently completely at rest).

There are two kinds of electrical activity which develop as a response involving chemical thermoregulation. The first kind is the well-known cold shivering, which takes place only after considerable cooling has occurred. The second kind consists of thermoregulatory tone, which is a more precise method of chemical thermoregulation. During a gradual transition from a raised body temperature to a very low one, thermoregulatory tone is initiated considerably earlier than cold shivering, and disappears earlier too.

It is clear that different physiological mechanisms underlie the two processes.

It appears that the muscular system plays a definite part in thermal regulation, even where extreme body

temperatures are concerned. Thus, for instance, the complete absence of electrical muscular activity during raised body temperature must be interpreted as an adaptive mechanism which reduces the formation of heat in muscle to a minimum. On the other hand, a certain degree of electrical activity is maintained in some muscles at very low temperatures. This result confirms the view of A. D. Slonim [6], A. Izbinskii [4] and Giaja [11] who maintain that the constant-temperature body mechanisms continue to operate even in profound hypothermia. It is interesting that the body's response to cooling is maintained even after the higher nervous centers have been put out of action. This effect is precisely the opposite of that which we observed in our experiments on hypoxia, when thermoregulation failed comparatively early, and muscular electrical activity was completely suppressed, although cerebral electrical activity was well maintained [2].

It is important to note that during progressive cooling, activity is maintained longest in the proximal parts of the body. It would appear that we are here concerned with a mechanism of "regional" thermoregulation which has the effect of maintaining the highest temperatures in the most important parts of the body. "Regional" thermoregulation occurs in all homothermic animals, and results in only certain of the organs and tissues (brain and viscera) having a constant temperature, while the temperature of the peripheral parts of the body varies greatly with the temperature of the surroundings [8, 12 and others]. Regional thermoregulation is particularly well developed in hybernating species on awaking from the winter sleep. During the warming up period, the temperature in the mouth may be 10-15° higher than that in the rectum (review and original observations by Eisentraut [10]). This interesting observation requires further study.

SUMMARY

Gaseous exchange and electrical activity of skeletal muscle were studied in white rats at rest. The rectal temperature varied between 38.5°-40° and 18-11°. It was shown that when the body temperature was high, gaseous exchange was minimal and there was no electrical activity in the muscles. When the body temperature fell, both gaseous exchange and muscular electrical activity first rose and then fell. At very low body temperatures, only the muscles of the proximal parts of the body continued to manifest some electrical activity.

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THE REFLEX NATURE OF THE PERIODIC GASTRIC HUNGER CONTRACTIONS

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Trnaslated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 23-28, November, 1960
Original article submitted January 18, 1960

Despite the large number of investigations of the nature of the periodic hunger contractions of the digestive system, much work remains to be done. The great majority of investigators consider them to be induced by some humoral influence acting both on certain central nervous structures [7, 14] as well as directly on the effector endings of the vagus and sympathetic nerves supplying the muscles of the gastrointestinal tract [1, 4, 6, 9, 12, 13, 16].

Carlson [15] associated the phasic activity of the empty stomach with the activity of the local nervous apparatus of the gastric wall. N. V. Raeva and L. K. Pupko [8], M. V. Tetyaveva [11], V. N. Ivanov [3], and N. N. Lebedev [5] attribute the periodic gastric contractions to the activity of the local nervous apparatus as influenced by a tonic inflow from the vagi.

The fact that the contractions are altered by division or by stimulation of the nerves supplying the stomach emphasises the importance of the part played by these nerves.

There are numerous references to the stimulating effect of the vagi on the activity of the empty stomach. However, the problem as to whether this activity is determined by naturally occurring afferent impulses passing along the vagi from the digestive tract has not previously been investigated.

In the present work we have determined the effect on the periodic gastric hunger contractions of the flow of afferent impulses reaching the vagal nucleus.

We have shown previously that it is possible to induce gastric contractions at periods when they would not normally occur, by artificially stimulating with sodium chloride solutions the receptors of the vagus after they had been associated with the skin by a nervous anastomosis with the vagus medianus. However, this method gave only indirect information concerning the parts played by afferent vagal fibers in the generation of gastric hunger contractions. The present work represents an attempt to establish directly the part played by the naturally occuring afferent vagal impulses in stimulating these contractions.

METHOD

The experiments were carried out on three dogs. All were supplied with gastric fistulae and a balloon, and the gastric contractions were recorded by the usual method. In addition, in each dog an anastomosis was made between the central end of the right vagus and the peripheral end of one of the nerves of the front legs [2]. The left vagosympathetic trunk in the neck was brought out into a strip of skin. In two dogs the skin strip contained the right vagosympathetic trunk above the site of anastomosis.

The left vagosympathetic trunk was contained in a portion of skin, and so could be blocked at any moment, and in all the animals the right vagus had been divided either mechanically or chemically. Mechanical block

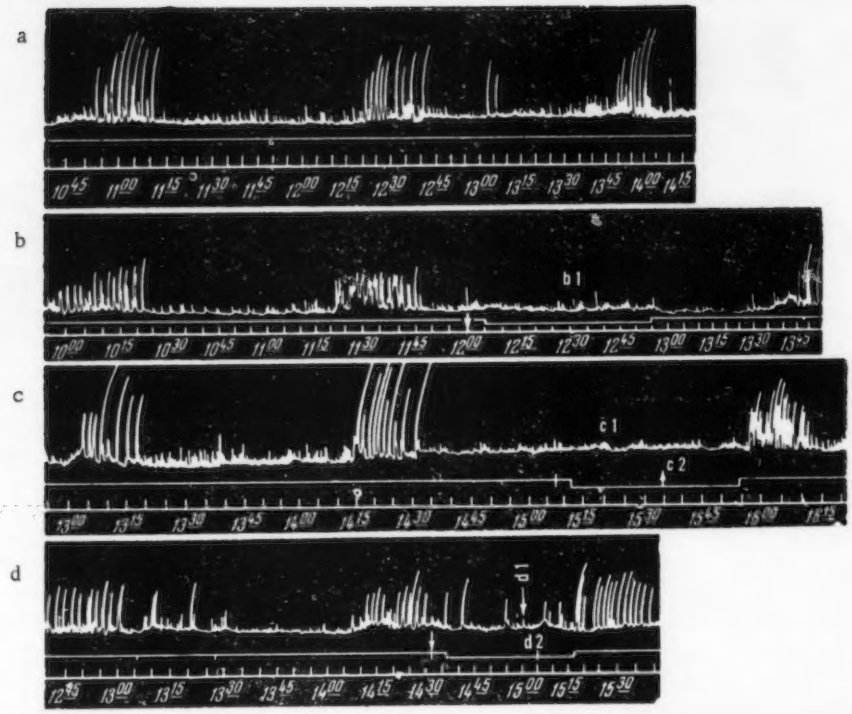

Fig. 1. Change in the gastric contractions following mechanical block of the remaining left vagosympathetic trunk. a) Initial rhythmic gastric hunger contractions; b) block established at the beginning of a resting period; c) block established at the end of a resting period; d) stimulation of the receptors of the right anastomosed vagus nerve, combined with block of the right vagus at the beginning of a resting period.
Key: b1) Reduction of the left vagus; c1) predominance of the left vagus nerve trunk; c2) P_S 200 in 1' Horner' syndrome; d1) Horner's syndrome from the left P_S 200 in 1'; d2) predominance of left vagus nerve trunk, 5% NaCl.

was made by applying pressure to the nerve by an inflated rubber cuff contained within a metal ring round the skin bridge. Usually a complete block occurred after 15-20 minutes of applying an air pressure of 120 mm mercury to the inside of the cuff. The onset of complete block was usually determined by observing the development of Horner's syndrome, and an increase of pulse frequency to 200 beats per minute.

Chemical block was induced by injecting 30 ml of 0.5% warmed novocaine solution into the nerve trunk. The dogs were starved for not less than 18-20 hours before the experiment.

RESULTS

First of all, in each animal the normal periodic hunger contractions were recorded. In two of them (Charley and Klyuchik) the active periods lasted on average for 15-20 minutes, alternating with periods of rest lasting for one hour (Fig. 1a). In the dog Ezhik, twenty-minute active periods were separated by intervals of one and a half hours.

In the first set of experiments a mechanical block was applied to the vagosympathetic trunk brought out into a strip of skin on the neck. It was found that the effect of such a block on the onset of gastric contractions depended on the moment of the resting period during which it was applied. If the pressure was applied at the beginning of a resting period, then the next active phase did not occur after the release of the block for a time equal to the length of a normal resting period (Fig. 1b). When a mechanical block was established in the middle of a resting period, the next active phase developed at a time after the release of the block equal to the difference

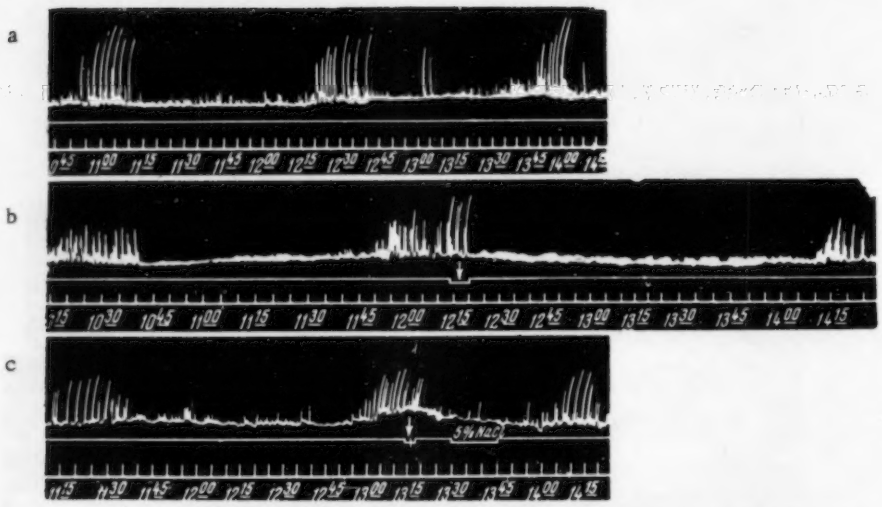

Fig. 2. Change in gastric motility in a dog following novocaine block on the left vagosympathetic trunk. a) Initial rate of gastric contraction; b) injection of 10 mg per kg of a 0.5% novocaine solution into the skin strip of the left side; c) stimulation of the receptors of the right anastomosed vagus by application of a 5% sodium chloride solution to the skin while applying a novocaine block to the left vagus.

between the normal duration of the resting period and the time which had elapsed between the end of the previous active period and the application of pressure to the nerve. However, when the block was established towards the end of a resting period and 15-20 minutes before the onset of the next contraction phase, the next period due occurred immediately after the block was removed (Fig. 1c).

It might be thought that one of the possible reasons for the delay in the operation of the mechanical block applied to the single remaining vagosympathetic trunk, when established at the beginning of a resting period, would be the stimulating effect of the block both on central nervous structures as well as directly on the gastric motor apparatus. However, the fact that mechanical block established at the end of a resting period did not prevent the development of hunger contractions from occurring after it had been removed, demonstrates in our opinion that such a procedure exerts no inhibitory effect on gastric contractions. All the same, special control experiments were arranged to check this point. In the dog Ezhik, the block was established on the trunk of the right anastomosed vagus which was brought out into a similar strip of skin. The left vagosympathetic trunk then remained intact. When the anastomosed vagosympathetic trunk was blocked, no changes were observed in either the type or the rate of the gastric contractions.

It seems to us that these control experiments exclude any possible direct stimulant action on a nervous center by the block itself. It must also be supposed that the stimulant effects of any mechanical stretching of the vagosympathetic trunk must have been prevented by the previous injection into it of 2 ml of a 2% novocaine solution, which was given in every case when the mechanical block was applied.

In our opinion the delay in the onset of the next set of gastric contractions which occurs when the block is applied at the beginning of a resting period was due to the arrest of afferent impulses passing along the vagus from the gastrointestinal trunk to the vagal center. As confirmation, it was decided to block the current of afferent impulses to the vagal nucleus during a resting period. For these purposes, during a block of the left vagosympathetic trunk the receptors of the right vagus were stimulated in the skin by a 5% sodium chloride solution. The effect was that a period of gastric contractions usually occurred immediately after withdrawal of the block (Fig. 1d).

These experiments have therefore shown that a mechanical block of the vagus established at the beginning of a resting period prevented the flow of afferent impulses to the vagal nucleus, which in turn prevented the return of excitability of the central vagal apparatus. It is only when the nerve block was removed, and when the

flow of afferent impulses to the center was restored that its excitability once more began to return to the critical point beyond which efferent discharges were distributed to the periphery. At the end of a resting period, the center had probably succeeded in becoming "charged" into its operating state, so that vagal block at this period prevented only the spread of efferent impulses to the periphery.

In the second set of experiments an attempt was made to eliminate the conduction of afferent vagal fibers during a certain time interval. For this purpose a novocaine vagosympathetic block was used; 10 mg per kg of a 0.5% solution of novocaine was injected into a portion of skin.

Control experiments showed that this procedure effectively produced a selective block of the afferent vagal fibers only. Thus, when the novocaine solution was injected into the portion of skin containing the right anastomosed vagus nerve, for the next 30-50 minutes it was not possible to obtain the normal reflex response to stimulation of the cutaneous vagal receptors which normally takes the form of coughing or vomitting in response to chemical stimulation [2]. However, the leg continued to move in conjunction with the act of swallowing and respiration, which showed that the continuity of the efferent fibers of the anastomosed vagus were intact.

When a 0.5% novocaine solution was injected into the strip of skin containing the single remaining intact left vagus nerve, there was a delay of 30-50 minutes in the occurrence of the next set of gastric contractions (Fig. 2b).

Special experiments showed that during this procedure the excitability of the vagal nucleus to stimulation of the receptors of the right anastomosed vagus was not changed. In response to stimulation of the receptors of the right vagus with 5% sodium chloride solution during novocaine block of the left vagus, a set of gastric contractions was initiated out of its normal turn (Fig. 2c). These experiments showed that the conductivity of the efferent fibers was maintained during novocaine block of the single remaining vagus nerve. It follows therefore that the observed delay in the occurrence of the next set of gastric contractions which follows novocaine block of the single intact left vagus may be due principally to suppression of conduction in its afferent fibers.

In our opinion the results indicate definitely that afferent impulses passsing along the vagal fibers during the resting period are concerned in the development of the periodic gastric motility.

On the one hand, by stimulation of the vagal receptors, which causes an additional flow of afferent impulses into the vagal center we have been able to cause a set of gastric contractions to occur out of their normal turn [10]; on the other hand, selective block of the vagal afferent fibers has been shown to cause a delay in the appearance of the contractions.

The part played by humoral influences is apparently of secondary importance. Indeed, if the periods of excitation of the vagal nucleus were induced by humoral factors, then when the efferent vagal fibers were conducting, gastric hunger contractions ought always to occur. But in fact they do not, as can be seen from the experiments described above.

When there is hunger, the humoral factors very likely determine merely the initial level of excitation of the central nervous structures. The periodic processes of excitation which occur in the vagal nucleus are elaborated under the influence of afferent impulses from the gastrointestinal tract passing to the center along the vagi.

SUMMARY

A study has been made of the mechanism of the periodic gastric hunger contractions. An anastomosis was made between the central portion of the right vagus and the peripheral end of one of the nerves of the forelimb. Cutaneous receptors of the right vagus nerve were stimulated; the left vagus nerve was brought out into a skin bridge on the neck, and a mechanical and a novocaine block was established. The results indicate that afferent impulses in the vagi are concerned in stimulating gastric hunger contractions.

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DIURESIS AFTER WATER IS GIVEN PER RECTUM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 28-32, November, 1960
Original article submitted March 30, 1959

It is usually thought that the increase in diuresis which follows a water load (water diuresis) is caused chiefly by a reflex originating in the receptors of the tissues and vessels, and brought about by changes in the secretion of the antidiuretic hormone of the hypothesis. (The mechanism of the osmoregulatory reflex has been studied in recent years in a laboratory directed by Professor A. G. Ginetsinskii.)

A large number of experimental results also indicate that reflex influences from the upper part of the digestive tract originating in the act of drinking itself also play an important part [2, 6, 7]. These influences are particularly significant in connection with the rapid onset of water diuresis. This circumstance may explain the numerous observations of the less definite diuresis which follows parenteral introduction of fluid [10, 11, 13].

It therefore seemed worthwhile to examine the process of diuresis following the administration of water rectally, a procedure which eliminates any reflex effects from the upper part of the gut.

METHOD

The work was carried out as chronic experiments on five dogs in which the ureters were exteriorized by the method of Pavlov-Tsitovich. The food ration was maintained as nearly as possible constant. It was given once per day, 16-18 hours before the beginning of the experiment. Before the experiment, the animals were taken for a walk in order to empty the lower part of the intestine.

A water load of 300 ml was given either as a drink, in which case 10% milk was added to the water, or rectally over a period of 15-20 minutes through a rubber catheter. The experiment lasted six hours, and urine was collected at half-hour intervals. Besides urinary excretion, measurements were made of filtration and reabsorption, and of the hydremic response to drinking water or receiving it rectally.

Filtration and reabsorption was determined from the endogeneous creatin. In addition to the actual values of filtration and reabsorption, the changes in these quantities were also significant. The hydremic response was determined from the dry residue, which according to many authors [5, 13, and others] represents the most direct and accurate indication.

In all, 71 experiments were carried out, in 28 the fluid was presented as a drink, and in 43 the water was given rectally. Filtration and reabsorption were studied in 41 experiments, in 19 of which the dry blood residue was determined. The above quantities were measured several times in each experiment, twice before the water load, and 4-6 times after it.

RESULTS

In th 28 experiments in which water was given by mouth, there was a large increase in diuresis which started within half-an-hour of drinking (Fig. 1). After 3 hours, diuresis again fell. The marked increase was

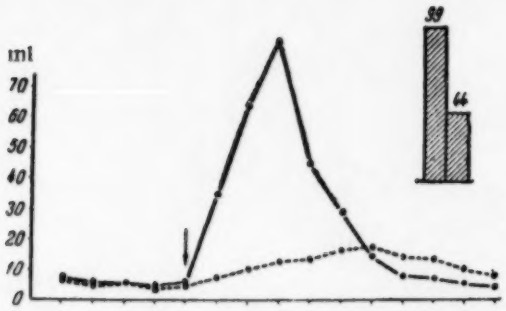

Fig. 1. Change in diuresis in the dog Belka after drinking 300 ml of water (continuous line represents mean of 12 experiments) and injecting the same amount into the rectum (dotted line represents mean of 14 experiments). The arrow indicates the moment at which the fluid is introduced. Abscissa – time (each division represents 15 minutes); ordinate – diuresis (in ml). The diuresis is shown as a percentage of the total fluid given, left column after oral, right after rectal administration.

due almost entirely to a reduced tubular reabsorption (Fig. 3), a result which has been repeatedly reported [4, 15, and others]. Filtration showed no very great changes, although in half the experiments the increase in the diuresis during the first half of the period after drinking was partially due to a noticeably increased filtration. In three of the 12 experiments the increased filtration was quite considerable, a result which agrees with the findings of several other authors [1, 3].

Of the 34 experiments in which water was introduced rectally, in three the water diuresis differed little from normal, and in eight there was a marked increase in the urinary excretion which was less well shown, and in 32 the water introduced rectally caused either no increase in diuresis or else one which was very weak and ill defined (Fig. 1). The lag in diuresis after water has been taken in rectally was particularly clearly shown during the first two hours after its administration, and occurred in all the animals (Fig. 2). In the few experiments when the water given per rectum caused a much more marked increased in diuresis, the increased excretion was due chiefly to a reduced reabsorption. It was however much less well shown than in the experiments in which water was taken orally. In the latter case, in almost all the experiments reabsorption was greatly reduced in the first hour after taking the fluid, whereas when it was given rectally the effect was observed only in one out of 28 experiments. During the second and third hour after giving water rectally, there was a marked reduction of reabsorption in only seven of the 28 experiments, whereas whengiven by mouth the reduction in the reabsorption was very marked and occurred in all cases.

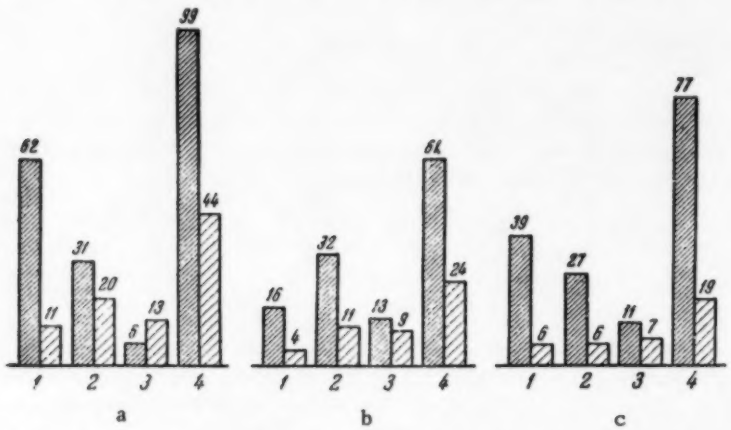

Fig. 2. Change in diuresis after a water load of 300 ml taken either orally (close shading) or rectally (sparse shading) as a percentage of fluid intake. 1) During first hour; 2) during second hour; 3) during third hour; 4) total over three hours; a) in dog Belka (mean of 26 experiments); b) in the dog Tainy (mean of 21 experiments); c) in the dog Diana (mean of 14 experiments).

When the water was given rectally, there was an increase in diuresis due to increased filtration in only four of the experiments. In the remainder, filtration was either unchanged or varied within the previous limits.

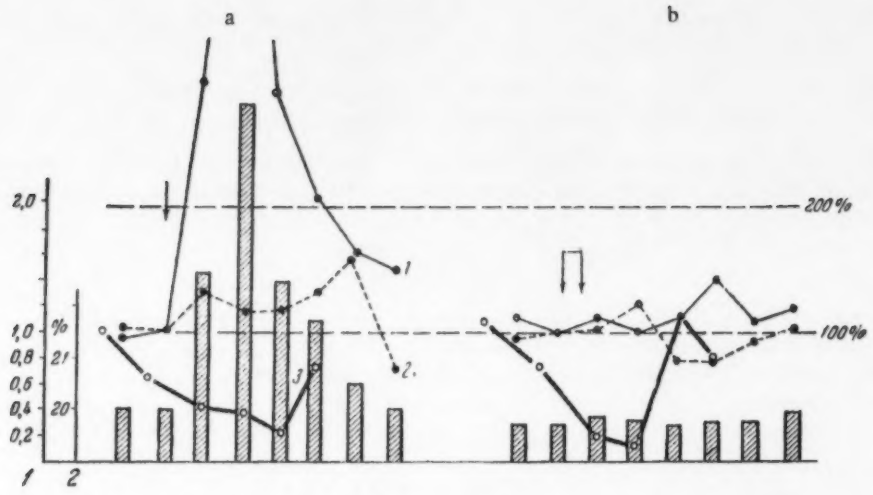

Fig. 3. Effect of a 300 ml water load in the dog Diana on filtration, reabsorption, diuresis, and dry blood residue when the fluid is taken (a) orally and (b) per rectum. Experiments Nos. 21 and 22. 1) Amount of filtrate not reabsorbed (excreted with the urine) as a percentage of the initial amount; 2) filtration (in ml per minute) as a percentage of the original value; 3) dry blood residue as percentage (the arrow indicates the moment at which the water is given, the columns the diuresis). Abscissa – time, 30 minutes intervals; ordinate: 1) diuresis (ml/minute); 2) dry blood residue.

Which ever way the fluid was administered, the hydremic reaction was shown to various extents.

Thus, for instance, in the dog Belka, the maximum reduction of the dry residue after drinking was 7.5%, and when the fluid was given rectally it was 7.8%. In Diana, the corresponding figures were 11.2 and 13.6%. However, in some experiments in which the water was given rectally, the hydremic reaction was less well shown than when it was taken by mouth. It must be noted that the hydremic reaction which occurred in most of the experiments did not follow the course of the diuresis. In some cases when there was a hydremic reaction diuresis was increased, and again, when there was no hydremic response a copious diuresis occurred. These observations correspond with previous reports [12, 14].

Our investigations have threfore shown that the increased diuresis which follows the rectal intake of fluid occurs much later and is much less marked than when the fluid is given by mouth. It appears that in the first case the indefinite diuresis is caused by a slow absorption of fluid from the rectum. This conclusion is however contradicted by the results on the hydremic response following rectal water intake. Also, we carried out additional experiments in which fluid was withdrawn from the rectum by means of a rubber tube introduced through the sphincters. Five minutes after water had been introduced, no more than 25% of the volume given could be recovered, and after 15 minutes no fluid could be withdrawn. A few experiments were also carried out in which pure water was drunk instead of the mixture of water and milk. The normal well-developed water diuresis then occurred. This result showed that the increased diuresis following water taken by mouth was not due to its milk content.

Some experiments were performed in which water was given by mouth and a catheter introduced into the rectum for 15-20 minutes, in order to exclude any possible influence of this procedure on diuresis; it was found not to reduce the diuresis in any way. In order to prevent as far as possible any influence on urinary excretion from distention of the large intestine, as described by N. A. Myasoedova [9], we used fluid which had been warmed to body temperature, and introduced it slowly. It must also be remembered that in our experiments the rapid absorption of the fluid introduced reduced the stretching of the intestine, whereas in N. A. Myasoedova's experiments the dilatation of the rectum was continued for several hours.

M. L. Linetskii [7] also observed an indefinite diuresis after introducing fluid into the small intestine of

patients with a jejunostomy and V. F. Lysov [8] observed the same effect following a water load introduced through a fistula of the large intestine.

This study allows us to conclude that the reduced diuresis following water introduced rectally is due to the absence of stimulation of the normal receptive field concerned in reflex diuretic stimulation.

The results may have some practical importance. For example, when it becomes necessary to introduce fluid into the body, it is better to do so rectally, whereas in order to stimulate a powerful diuresis it should be given by mouth.

SUMMARY

Water given rectally to dogs with ureteric fistulae caused a much smaller diuresis than when the same quantity of fluid was given by mouth. Reabsorption was also decreased but to a smaller extent, while filtration remained unaltered. The hydremic reaction did not run parallel to diuresis whichever way the fluid was taken. The results obtained confirm the importance in water diuresis of reflex stimuli originating in the upper parts of the digestive tract.

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THE RELATIONSHIP BETWEEN THE LOCALIZATION OF RADIATION INJURY AND THE FUNCTIONAL STATE OF AN ORGAN

COMMUNICATION 2

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 33-36, November, 1960
Original article submitted December 22, 1959

In a previous investigation [9] we showed that the degree of manifestation of a radiation injury of the kidneys and its localization are determined by the magnitude and character of the functional load on the nephrons at the moment of irradiation. An increase in the renal function during the period of exposure to ionizing radiation causes the radiation lesion to be localized mainly in the parenchyma of the kidney. The strict regularity of this relationship is firm evidence that the state of increased function intensifies the degree of manifestation of radiation injury to an organ. These observations enabled us to employ the same experimental model, with slight variation of the experimental conditions, in order to attempt to solve certain problems of the pathogenesis of radiation sickness. We wished to find out whether the character of the course of radiation sickness is determined during the actual period of exposure to the radiation or whether the "after-action" period is of decisive importance. It was a working hypothesis in the investigation that, if a functional loading of the kidneys, applied in the period of development of radiation sickness, produces a more severe radiation injury of the nephrons, this is proof of the greater importance of the period of establishment of radiation sickness. In the event of the converse results of the investigations – absence of increased severity of injury to the renal parenchyma – it could be considered that the degree of structural change of the organ and the character of the course of the disease are determined purely by the direct action of ionizing radiation on the animal body.

METHOD

The investigations were conducted on sexually mature C57 pure line mice, weighing 22-28 g. About 350 animals of both sexes were under observation. For single whole-body irradiation of the mice we used an RUM-3 apparatus under the following conditions: voltage 180 kv, current 10 ma, filter 0.5 mm Cu + 1 mm Al, dose rate 45-48 r/min, focus distance 30 cm, dose 1400 r (measured in the air with a GRI dosimeter). During irradiation each mouse was placed in a separate cardboard box, measuring 7 x 5 x 4 cm. The experimental and control animals were irradiated simultaneously. On the first, second and third days after irradiation the experimental animals received intraperitoneal injections of euphyllin. The size of each dose of euphyllin (1.2 mg of the standard preparation in ampules, in 1 ml water) was determined experimentally; it produced increased diuresis without causing morphological changes in the renal parenchyma. We measured accurately the survival period of the experimental and control animals after irradiation; we made macro- and microscopic studies of the material, using various histological methods for this purpose.

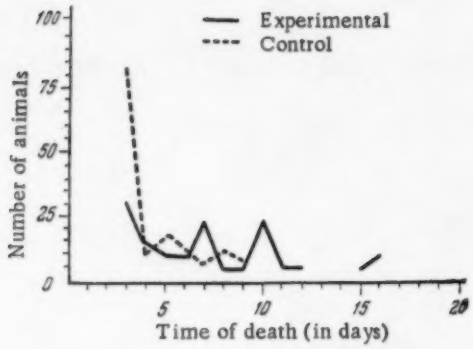

Fig. 1. Duration of life of experimental and control animals.

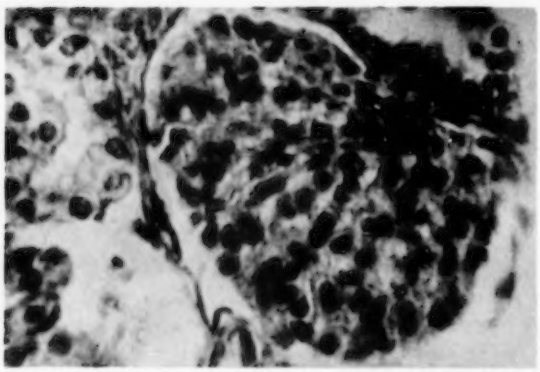

Fig. 3. Enlargement of the glomerulus and increase in number of its nuclei. Photomicrograph. Ocular 12×, objective 45×. Stained with eosin-hematoxylin.

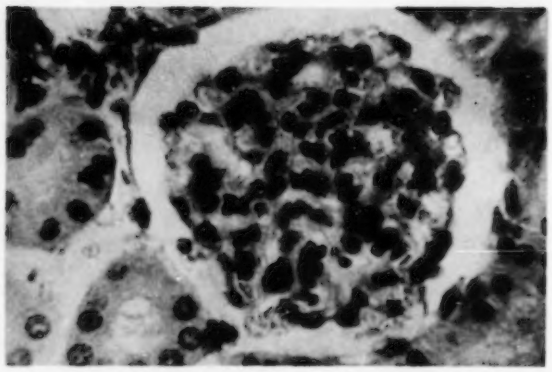

Fig. 2. The congested glomeruli retain the structure of the vascular network. Photomicrograph. Ocular 12×, objective 45×. Stained with eosin-hematoxylin.

RESULTS

All the experimental and control animals died from radiation sickness, but there were certain differences in the duration of its course and in the severity of the morphological changes in the kidneys. The survival period of the control animals varied from 3 to 9 days; their mean duration of life was 4.35± 0.43 days. Considerable uniformity of the experimental material was found; in the great majority of cases the mice died on the third day. This can be seen clearly in the diagram, which is characterized by a single sharp fall on the curve (Fig. 1). Death of the experimental animals took place between the third and 16th days, although in one case, which we excluded from the statistical treatment of the results, death was observed even 30 days after irradiation. The mean duration of survival of the experimental animals was 6.8± 0.66 days. This group of cases showed considerable variation in the survival period of the mice, giving a drawn-out curve with many peaks (see Fig. 1). It must be emphasized that in almost every case the duration of survival of the animals receiving euphyllin was longer than that of the control animals. The only exception was one case in which the control mouse lived longer than the experimental mouse and no radiation injury was present in the kidneys.

The macrosopic material was characterized by great constancy of the morphological changes, which were typical of acute radiation sickness. Emaciation was always marked, more so when the survival period of the animals was prolonged, and was accompanied by a hemorrhagic diathesis and atrophy of the hemopoietic tissue. If the mice died early the predominant changes were those typical of radiation injury in the intestine.

In the experiment on mice receiving the diuretic after irradiation, in the great majority of cases (98%) the typical morphological changes of radiation injury were absent from the renal parenchyma. When the animals died at an early period (between 3 and 5 days) the glomeruli were distinguished by severe congestion; their vascular network retained its clear pattern, and the endothelium was well defined (Fig. 2). The structure of the tubules remained unchanged. With a longer period of survival of the mice after irradiation (over 6 days) large tubules with a high proportion of nuclei were often seen (Fig. 3). The mean diameter of the tubules in the experimental animals was 53± 1.7μ and in the controls 49± 1.6μ. Many nuclei of the enlarged tubules showed hyperchromatosis and were of a considerable size. The epithelial portions of the nephrons retained their usual structure. In three cases radiation injury of the kidneys was also observed in the group of experimental animals; its morphological picture was restricted, however, to a slight swelling and basophilia of the walls of the vascular network of the glomeruli and to signs of necrobiosis in the individual nuclei. Identical changes could also be seen

in certain arterioles of the interstitial tissue of the kidneys. Foci of necrobiosis were present in the epithelium of the main divisions of the tubules.

In all but one of the control animals (0.7%) the typical picture of radiation injury was seen. The walls of the arterioles of the renal glomeruli and interstitial tissue were very swollen, homogeneous and basophilic. In many vessels the endothelium was in a state of necrobiosis. In the epithelium of the tubules there were marked dystrophic changes, turning into necrosis. In some cases the lumen of the tubules contained numerous cylinders. Small hemorrhages were often present.

In order not to overload the articles with factual matter, we shall state only that identical results were obtained in experiments in which caffeine was used as the diuretic. The administration of mercusal (a mercurial diuretic) to irradiated animals led to an obvious shortening of their period of survival and greatly increased the morphological signs of radiation injury to the kidneys. The observations are in full agreement with the pharmacological data that mercusal may only be used in the absence of kidney disease [1], otherwise the toxic diuretic will only aggravate the pathological changes in the organ.

The results of our experiments were thus somewhat unexpected, and in some ways did not correspond to the original idea of the investigation. The increased functional load on the kidneys after irradiation brought about a statistically significant ($t = 3.175$) lengthening of the period of survival of the irradiated animals and almost abolished the morphological changes in the renal parenchyma. The prolongation of the course of the radiation sickness was accompanied by a change in its morphology. The bulk of the control animals showed the "intestinal" form of radiation sickness, which, according to the literature [7, 9, 10, 11], is characteristic of the action of massive doses of ionizing radiation. In the experimental animals other forms of manifestation of radiation sickness predominated, the main localization of the typical morphological changes being in other organ systems, primarily in the hemopoietic tissue. In other words, in these cases the character of the morphological signs of the disease corresponded to the usual pattern of injury resulting from exposure to much smaller doses of irradiation. In these experimental conditions, therefore, the administration of diuretics caused some decrease in the severity of the manifestations of radiation sickness.

Comparison between these facts and the results of previous investigations shows, in our opinion, that the direct, injurious action of ionizing radiation is of great importance in the pathogenesis of radiation sickness. At the same time, however, it is impossible to ignore the role of the "after-action", an important element of which is evidently the toxic products of tissue destruction. The excretion of these "toxic factors" [4, 5, 6, 8] by the kidneys, functioning at a higher level of intensity, was also probably responsible for the sufficiently demonstrative results of these various investigations.

Our observations differ from the results of work carried out in S. S. Vail's laboratory [2, 3]. In these investigations an increase in the severity of the radiation pathology was observed when the function of the heart and gastro-intestinal tract was loaded after irradiation of the animals. The obtaining of contrary results from similar experiments evidently depends on essential differences in the character of the function of the organ system studied. The favorable influence of increased work of the excretory organ on the course of radiation sickness illustrates well the role of toxic substances in the pathogenesis of the disease. It can be concluded from this observation that the course of radiation sickness may be actively influenced, in the sense of decreasing the severity of its manifestations, by the repeated administration of diuretics acting upon the vascular elements of the nephrons.

SUMMARY

An attempt was made to study the effect of increased diuresis, induced following irradiation, upon the morphology of the kidneys. Euphyllin was repeatedly administered to mice of the C57 lineage following general irradiation (apparatus RUM-3, voltage – 180 kv, current – 10 ma, filter – 0.5 mm Cu + 1 mm Al, intensity – 45-48 r/m, dose – 1,400 r). Euphyllin administration considerably prolonged the animals' life span and protected the kidneys from radiation injury. The effect of a diuretic is regarded by the authors as being due to the excretion of the toxic products of tissue disintegration (appearing as a result of the action of ionizing radiation).

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EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN DOGS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 37-41, November, 1960
Original article submitted March 31, 1960

In the experimental study of atherosclerosis during the past few decades, many experiments have been undertaken to produce atherosclerosis by N. N. Anichkov's method, by means of the prolonged feeding of cholesterol to rabbits and fowls. Recently experimental atherosclerosis has also been produced in dogs [1-8]. According to existing evidence, this last model resembles human atherosclerosis closest in the character and localization of the morphological changes in the cardiovascular system. In dogs, even in the earliest stages of development of cholesterol atherosclerosis, the coronary arteries of the heart and the vessles of the base of the brain are involved, i.e., those portions of the cardiovascular system which suffer most often in atherosclerosis in man.

The present work is devoted to the study of certain problems of the clinical physiology of atherosclerosis in experiments on dogs.

METHODS

Most authors [1-3, 5-8] have produced experimental atherosclerosis by the enteral administration to dogs of a solution of cholesterol in fat or ether, together with methylthiouracil. As a preliminary step, 2-3 months before receiving the cholesterol, the dogs were given methylthiouracil in order to suppress thyroid gland function and thereby to lower their basal metabolism, since otherwise atherosclerosis does not develop in dogs. The thyroidectomy which is sometimes performed for this purpose is not always effective, for dogs often have accessory lobes of the thyroid gland.

In our work we used T. A. Sinitsyna's method [4], by means of which marked atherosclerosis may be obtained in a shorter time and without the preliminary administration of methylthiouracil alone. To ten male dogs, 3-4 years old and weighing from 16 to 22 kg, we gave a 20% solution of cholesterol in sunflower oil enterally for 120 days in a daily dose of 1 g cholesterol per kg body weight. Simultaneously with the cholesterol, for 60 days the dogs were given 1.5 g and, for the next 60 days, 1.0 g of 6-methylthiouracil. After the administration of cholesterol and of 6-methylthiouracil had been discontinued, the animals remained under observation for not less than one month. Two dogs which received 1.5 g 6-methylthiouracil daily, enterally, for 90 days acted as controls. During the first 60 days the dogs received the cholesterol solution and the 6-methylthiouracil in 200 g of boiled, minced meat. At the end of this period the majority of animals began to refuse this mixture and we had to give the same doses of cholesterol and 6-methylthiouracil forcibly. The experimenter opened the dog's mouth and his assistant dropped the cholesterol solution inside, without the use of a tube, after which the weight sample of 6-methylthiouracil was introduced far back into the mouth and the animal was given meat.

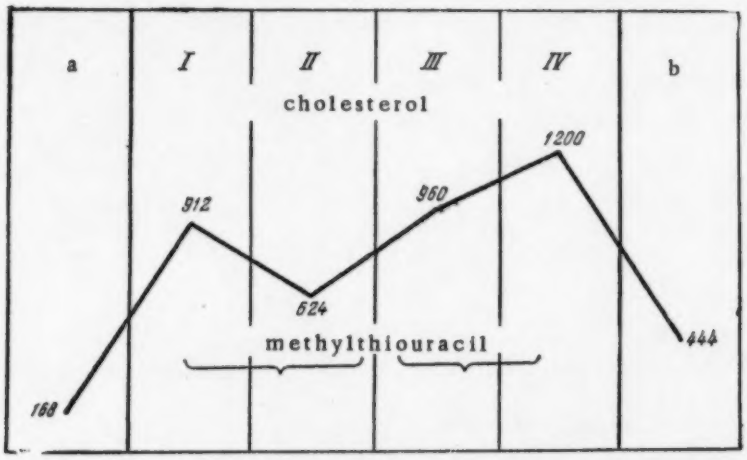

Fig. 1. Blood cholesterol concentration in dog No. 8. a) Initial concentration (mean of two determinations in the course of a month); I-VI) cholesterol concentration during feeding with cholesterol (1 g/kg body weight) and 6-methylcholesterol, by months; b) cholesterol concentration one month after cessation of feeding.

RESULTS

A few weeks after the beginning of the experiment the dogs became lethargic and made no playful movements. Some dogs grew thin and weak, and it was necessary to reduce their dose of 6-methylthiouracil or to discontinue it completely for 7-14 days. In the dogs of the control group there were no pronounced signs of hypothyroidism. The weight of the animals changed appreciably. In five dogs it rose at the end of the experiment by 2-5 kg, and in dog No. 8 it rose by as much as 10 kg. In three animals at first it rose by 1-2 kg, and then, as signs of hypothyroidism developed, it fell to 1.5-4 kg below the initial weight. The control dogs gained 1-2 kg in weight. One month after the cessation of administration of cholesterol and 6-methylthiouracil, the weight of the experimental animals almost returned to its initial value. The overloading with exogeneous cholesterol in conjunction with 6-methylthiouracil evidently caused considerable disturbance of the metabolic processes of the animal, more so than the administration of 6-methylthiouracil alone.

The blood cholesterol concentration was determined regularly, not less often than once every 3 weeks, in all the animals by Grigaud's method. Blood for the determination was taken from a vein in the region of the springing joint, always before the dogs had taken food, cholesterol and 6-methylthiouracil. The initial concentration of cholesterol in the blood varied in the experimental animals from 120 to 160 mg%, and rose in the course of the experiment on the average six- or eight-fold. The maximum blood cholesterol concentration in one dog reached 1200 mg% (Fig. 1). In the control dogs the blood cholesterol increased by 50-100%. In Fig. 1 a fall may be seen in the alimentary hypercholesteremia, taking place in the majority of dogs after 2-3 months of continuous administration of cholesterol and 6-methylthiouracil. This fact is evidently associated with the mobilization of the defensive and compensatory mechanisms of the body.

In all the dogs in the period of preliminary investigation, under ether and chloroform anesthesia, we exteriorized the left common carotid artery in a skin flap for measurement of the blood pressure and registration of the pulse wave. We measured the maximum arterial pressure by a palpatory method using a small rubber cuff, connected to a sphygmomanometer. The blood pressure was measured regularly at the same time of morning in a conditioned reflex chamber, before and after physical exertion in each experiment. During this procedure the dogs lay quietly on the bench without being strapped. The measurements began 1-3 months before the beginning of the experiment and continued for not less than a month after cessation of feeding. The maximum arterial blood pressure varied quite sharply from experiment to experiment; no animal in either the experimental or control group showed a persistent increase in the pressure. The reaction of the blood pressure to moderate physical exertion (running on an electrical moving belt for 5 minutes, with the belt moving at a velocity of 6.5 km/hr)

Fig. 2. Atherosclerosis of the branches of the coronary arteries of the heart (naked-eye specimen).

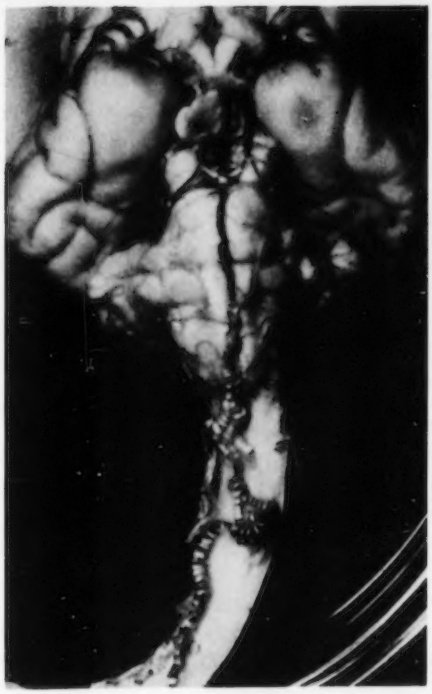

Fig. 3. Atherosclerosis of the arteries of the base of the brain and of the vertebral artery (naked-eye specimen).

differed in the dogs of the control and experimental groups. In the dogs of the control group the blood pressure after running on the moving belt usually fell slightly, and the heart rate showed hardly an increase. In the dogs of the experimental group after physical exertion the maximum arterial blood pressure rose moderately and the heart rate increased. It is evident that the adaptive reaction of the cardiovascular system to physical exertion follow a different course in healthy dogs and in dogs suffereing from atherosclerosis. In the healthy dog, in response to physical exertion the blood vessels dilate, especially the vessles of the working muscles. As a result, the peripheral resistance to the blood flow is diminished and the blood pressure falls slightly. In the dogs of the experimental group, with blood vessels affected by atherosclerosis, the functional and adaptive dilatation of the vessles in response to work is evidently inadequate and the next link in the adaptive reaction is brought into action– tachycardia and an increase in the arterial pressure, increasing the minute volume and ensuring an adequate blood supply during work.

Other authors [1, 2] have also drawn attention to the considerable lability of the blood pressure in dogs throughout the period of experiments.

One month after the end of the experiment the animals were sacrificed and subjected to pathologo-anatomical and histological examination. Investigation of the internal organs, vessels and brain of all the dogs receiving cholesterol and 6-methylthiouracil showed widespread changes in the vessel walls in the form of atherosclerotic plaques, present in various stages of development (from stains, hardly raised above the surface of the intima, to large formations deforming the vessel).

The largest number of plaques was found in the arteries of medium and small caliber, and also in the larger arteries, especially the carotid and femoral, the arteries of the base of the brain and the branches of the coronary arteries of the heart (Figs. 2 and 3).

No formed plaques were found in the wall of the aorta, but the intima of the aorta was slightly yellowish in places. These changes in the vessel walls were present to the most marked degree in dog No. 8, and were

comparatively slight in dog No. 7. Considerable accumulation of fat was observed in the liver. The medullary substance of the kidneys was greyish-yellow in color.

Microscopic investigation revealed marked lipoidosis of the internal organs. A large quantity of fat was found in the liver (in the parenchymatous and Kupffer cells), in the spleen (lipoidosis of the red pulp) and in the kidneys (the tubular epithelium). In the brain a small quantity of fat was present in certain cells in the capillary walls. In the wall of the aorta, in spite of the absence of gross macroscopic signs of disease, extensive foci of fatty infiltration were observed. The same diffuse fatty infiltration, together with macroscopically visible plaques, also took place in arteries of large and medium caliber.

In the dogs of the control group no changes were found in the cardiovascular system. The thyroid glands of both groups of dogs had the typical microscopic structure following administration of 6-methylthiouracil. Our experiments showed no clear parallel between the magnitude of the hypercholesteremia, the change in weight and the degree of the anatomical lesions of the vessels due to atherosclerosis.

Only in one dog was a combination of maximum blood cholesterol concentration, maximum growth in weight and most pronounced and widespread atherosclerotic changes in the cardiovascular system found to be present.

SUMMARY

No direct relationship existed (except in one case) between the weight gain, blood cholesterol content and the degree of morphological changes developing in the cardiovascular system of cholesterol and 6-methylthiourcil fed dogs. The localization and character of morphological changes in the cardiovascular system of dogs with experimentally induced atherosclerosis are similar to those in human atherosclerosis. The blood pressure in healthy dogs does not increase in response to moderate physical exertion; this is evidently due to reduction of the peripheral resistance as a result of adjustive dilation of the vessels.

The blood pressure in atherosclerotic dogs showed a slight rise in response to moderate physical exertion with concurrent tachycardia.

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FAT EMBOLISM OF THE THEBESIAN VESSELS*

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 41-46, November, 1960
Original article submitted December 12, 1959

Mechanical trauma is often followed by fat embolism. I. V. Davydovskii [4], for instance, states that an "aclinical" form of fat embolism is an almost constant background of any injury, whether a surgical operation or a war wound.

In fat embolism considerable lesions of the heart are often present. These may be revealed, in particular, by the electrocardiographic changes observed in fat embolism, the origin of which is associated by some authorities [13] with reflex spasm of the coronary arteries. The brilliant research of S. A. Vinogradov [3] has confirmed the fact that there is oxygen lack in the myocardium in fat embolism. These changes in the myocardium may be associated with disturbance of the coronary blood flow. Nevertheless, in the accessible literature we found no information regarding the role of the Thebesian vessels in trauma or the possiblity of fat embolism of these vessels.

In the present investigation our aim was to reproduce experimentally the entry of fat and ink from the right ventricle of the heart into the myocardium along the Thebesian vessels.

Experiments were carried out on ten dogs, weighing from 9.5 to 24 kg. In order to reproduce embolism we used fat and Chinese ink. The animals were anethetized with morphine and urethane, and an artificial respiration machine was used in the experiments. A cannula with a rubber tube leading to a flask was inserted into the left pulmonary artery. The right pulmonary artery was ligated. This was necessary in order to exclude the lesser circulation and to prevent any possibility of the passage of emboli through the orifice of the coronary arteries and the left ventricular vessels of Thebesius. Variants of this method were widely used in the well-known investigations of Pratt [12], Wiggers [15], and other authors. We injected either sunflower oil, heated to 38°, or Chinese ink, diluted with Lokce's solution, into the external jugular vein. The duration of contraction of the heart from the beginning to the injection of sunflower oil and Chinese ink was from 20 seconds to 7 minutes (the experiments were conducted in the Department of Normal Physiology). The dogs' hearts were removed from the thorax and fixed in a 10-15% solution of formalin. The weight of the heart, when full of oil or ink, varied from 180 to 300 g. The thickness of the wall of the right ventricle was 3-7 mm, and that of the left – 14-17 mm. Numerous orifices of the Thebesian vessels were found in the endocardium on the left and right ventricles. From the hearts, 33 pieces were excised for histological examination, in the endocardium of which Thebesian vessels were found. In this way we investigated areas of the efferent and afferent tracts, the region of the apex and the interventricular septum of the right ventricle. Several pieces of heart tissue were taken from the left ventricle. In this way serial sections were prepared for histological investigation. The sections were stained with hematoxylin, eosin, picrofuchsin, elastin and Sudan III.

*Given at a meeting of the Riga Society of Pathologoanatomists, September 20, 1957.

Fig. 1. Ink in the region of the orifice of a right ventricular Thebesian vessel. Ocular 7×, objective 20×. Stained with hematoxylin and eosin.

RESULTS

The experimental results are given in the Table, from which it can be seen that some relationship exists between the depth of penetration of the emboli along the right ventricular Thebesian vessles and the duration and strength of the contraction of the heart. In experiment No. 6, for instance, with weak cardiac contractions lasting 20 seconds, no emboli were found in the right ventricular Thebesian vessels. In experiments Nos. 1 and 2, with cardiac contractions lasting 30 seconds, emboli were found only in the region of the orifices and in the subendocardial sinusoids. In experiment No. 5, with energetic contractions of the heart lasting 4 minutes after the beginning of the experiment, emboli were found in the Thebesian vessels in the whole thickness of the right ventricular myocardium and in the right ventricular capillaries, arteries, arterioles and veins.

On microscopic investigation hemorrhagic foci and areas of fragmentation in the form of numerous transverse ruptures of the myocardium, affecting from two to eight muscle fibers, were found. The edges of the tears were uneven and displaced. In some sections Thebesian vessels were found opening out on to the endocardium of the centricules. According to work done in the Department and to our personal observations, the Thebesian vessels of the right ventricle are constructed in the manner of sinusoids. The latter were lined with endothelium, situated on a fine, barely perceptible underlying layer of elastic and collagen fibers. In the lumen of the right ventricular sinusoids were found erythrocytes, fat droplets and ink. We found both fat and ink in the region of the orifices of the sinusoids, in the subendocardial and more deeply situated sinusoids, and also in the arteries and veins of the myocardium (Fig. 1).

The histological sections thus gvave evidence of the presence of emboli not only in the orifices of the Thebesian vessels, but also in their deep branches and also in the arteries and veins of the myocardium.

It must be pointed out that there is a vast literature on the subject of embolism of the vessles of the heart. In particular, the vascular disturbances of the myocardium in cases of coronary embolism have been widely discussed in the classical textbooks and in articles in the current journals [10]. Nevertheless, in the accessible literature we found no description of embolism of the Thebesian vessels.

Our experiments to reproduce embolism of the Thebesian vessels of the right ventricle may widen our ideas of the circulation of the blood in the myocardium itself.

The experiments have demonstrated the possibility of access of blood from the right ventricle into the thickness of the myocardium. We account for embolism of the arteries and veins by the presence of anastomoses between the Thebesian vessels and the arteries and veins of the myocardium, which have been described by several authors [5, 6, 8, 11, 14].

Relationship between Depth of Penetration of Emboli and Strength of Cardiac Contractions

Experiment No.	Weight of dog (in kg)	Sex	Volume of oil injected (in ml)	Volume of ink injected (in ml)	Duration of injection of oil or ink	Duration of cardiac contraction from beginning of injection	Localization of emboli in Thebesian vessles of ventricles					Localization of emboli in arteries, veins and capillaries of the ventricles of the heart		Remarks
								right		left				
							orifice	subendocardial	deep	subendocardial	deep	right	left	
1	19	♂	–	20	28 sec	30 sec	+	+	–	–	–	–	–	Weak cardiac contractions
2	21	♂	–	60	28 sec	30 sec	+	+	–	–	–	–	–	Energetic cardiac contractions
3	15	♀	–	40	30 sec	1 min	–	–	+	–	–	+	–	Weak cardiac contractions
4	9.5	♂	–	70	3 min 51 sec	4 min 16 sec	–	+	+	–	–	+	–	Energetic cardiac contraction. After 5-6 contractions of the heart the branch of the left pulmonary artery was clamped for the duration of 3-4 cardiac contractions
5	14	♂	–	150	4 min	4 min	–	+	+	–	+	+	–	Energetic cardiac contractions
6	24	♀	20	–	20 sec	20 sec	–	–	–	–	–	–	–	Weak cardiac contractions
7	20	♂	70	–	1 min 2 sec	1 min 17 sec	–	+	+	–	–	+	+	Energetic cardiac contractions
8	18	♂	100	–	1 min 44 sec	2 min 44 sec	–	+	+	–	+	+	–	Energetic cardiac contractions
9	11.2	♂	100	–	3 min	4 min	–	+	+	–	–	+	–	Energetic cardiac contractions
10	13.5	♂	84	–	5 min	5 min	–	+	+	–	+	+	–	Energetic cardiac contraction. After 5-6 contractions of the heart the branch of the left pulmonary artery was clamped for the duration of 3-4 cardiac contractions

Fig. 2. Fat emboli in the lumen of the right ventricular Thebesian vessels. Ocular 15×, objective 8×. Stained with Sudan III.

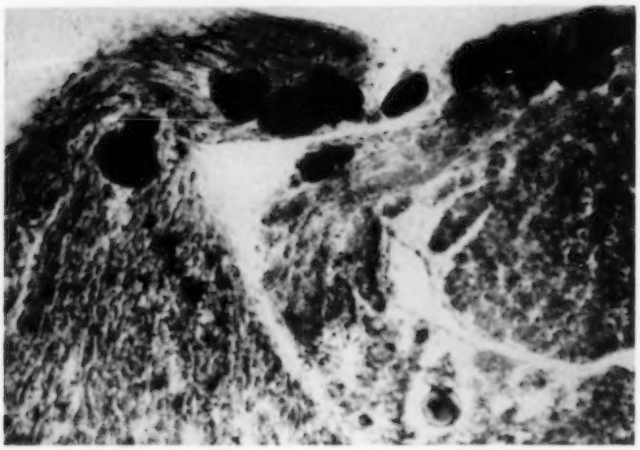

Fig. 3. In the region of the orifice and the subendocardial ramification of a right verticular Thebesian vessel there are fat droplests. Ocular 6×, objective 7×. Stained with Sudan III.

We therefore consider that the hemorrhagic foci and the areas of fragmentation of the myocardium may be associated with ambolism of the branches of the coronary system, which the emoli reach along the Thebesian vessels of the right ventricle.

A similar possibility of fat embolism may occur in clinical practice in cases of trauma.

We have made detailed studies of two cases of death from acute trauma in which we found fat embolism of the Thebesian vessels of the right ventricle in human subjects (Figs. 2 and 3).

In both the experiments and the cases of trauma in man, with fat embolism of the Thebesian vessels of the right ventricle we observed foci of dystrophy and necrosis of the myocardium. These changes are in agreement with the descriptions given by various authors working on the subject of fat embolism. In particular, I. V. Davydovskii [4] states that cardiac failure occupies an important place as a cause of death in fat embolism.

Functional changes in the heart in fat embolism have been recorded by clinicians by means of electro-

cardiography. The morphological changes under these same circumstances consist of hemorrhages beneath the endocardium along the course of the conducting system of the heart, and of fatty dystrophy of the muscle fibers [7]. In cases of fat embolism, S. A. Vinogradov [3] describes interstitial and perivascular edema, disappearance of the cross-striation of the muscle fibers, breaking up of their protoplasm into clumps, and small and extensive foci of necrosis. Side by side with these changes, a cellular reaction and sclerosis of the stroma were seen.

The pathogenesis of the changes described in the heart in fat embolism has been interpreted in various ways. Scherf and Schönbrunner [13] and others, for instance, explain them by reflex spasm of the coronary arteries. S. A. Vinogradov [3] associates the changes in the myocardium with discoordination between the work of the heart and its oxygen supply. A. I. Abrikosov [1], K. D. Éristavi [9] and others believe it possible that a direct obstruction of the branches of the coronary vessels with fat emboli lies at the basis of the changes in the myocardium.

From our point of view, in the interpretation of the microscopic changes in the myocardium associated with general trauma, it is essential to take account of the possibility of fat embolism of the coronary vessels of the heart and of the system of the Thebesian vessels.

SUMMARY

Embolism of the right ventricular Thebesian vessels was induced with fat and China ink in experiments on 10 dogs with the pulmonary circulation excluded. Thebesian vessels of the right ventricle were investigated in 2 patients, dying of trauma. Microscopic investigation of cardiac tissues in experimental and clinical conditions has demonstrated the presence of emboli in Thebesian vessels throughout the whole myocardium of the right ventricle, its capillaries, arteries, arterioles, and veins. These data point to the possibility of the blood from the right ventricle to enter the myocardium. One may, therefore, associate the presence of hemorrhagic foci and areas of myocardial fragmentation with embolism of the branches of the coronary system, where the emboli pass through the Thebesian vessels of the right ventricle.

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THE PROGRESS OF DYSTROPHIC CHANGES WITHIN THE NERVOUS SYSTEM (AS SHOWN BY VITAL STAINING)

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(Presented by Academician V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 46-51, November, 1960
Original article submitted December 29, 1959

The study of the mechanisms of development of a nervous dystrophy in association with various forms of injury to the nervous system is of great importance for the analysis of the pathogenesis of many diseases and manifestations of compensatory reactions of the body, aimed at restoration of the deranged functions.

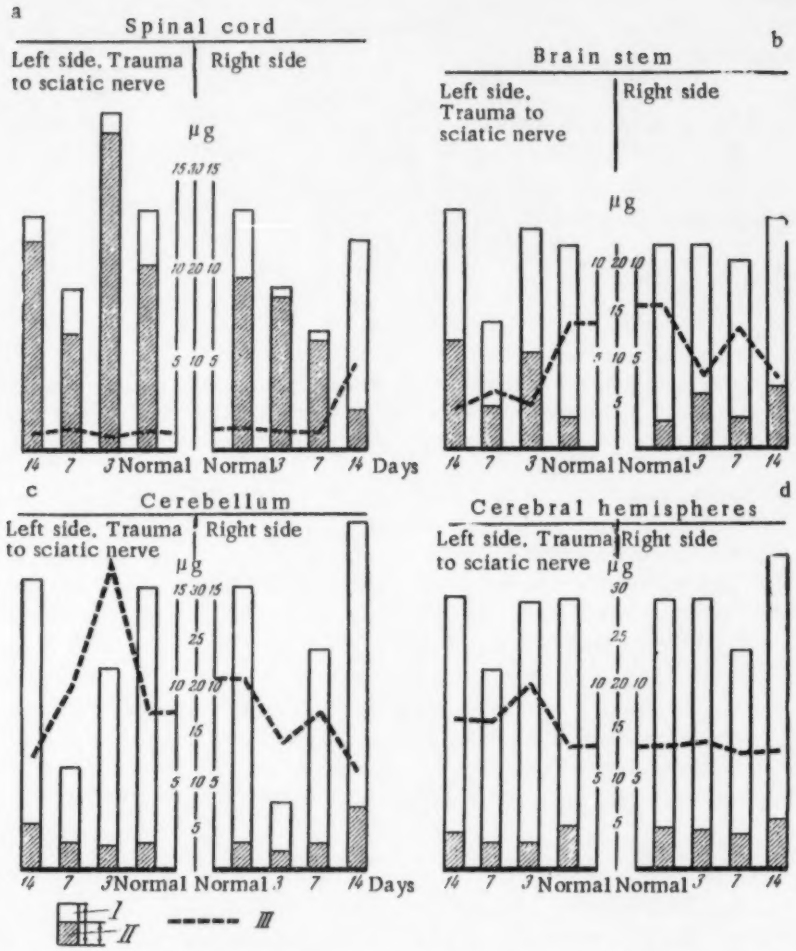
From a large volume of factual evidence, A. D. Speranskii [5] discovered a series of laws governing the character and the stages of development of a nervous dystrophic process in the peripheral tissues; at the same time he directed attention to the changes arising under these conditions in the various divisions of the nervous system itself.

The progress of dystrophic changes within the nervous system [5] has been the subject of detailed neurohistological investigations of the peripheral and central division of the nervous system. Work by B. S. Doinikov [3], M. L. Borovskii [2] and their co-workers has revealed both specialized and regular forms of lesion in the various nerve formations.

In summing up these findings, A. D. Speranskii [5] concluded that specialized and standard forms of dystrophic changes could take place in the nervous system. He emphasized the necessity of taking into consideration not only the properties of the pathogenic stimulus, but also the properties of the pathogenic stimulus in changing the state of the tissue by a reflex means. This is shown by the fact that the histopathological changes developing in the nervous system itself are not continuous in character and may be localized beyond the bounds of the segment in which the nervous trauma has been applied. In Speranskii's words [5], the pathological process "leap-frogged" over extensive and entire regions of the nervous system.

Of particular importance were experimental findings showing the asymmetrical character of the lesions in the various nerve formations, both in the same segment and in the more remote areas of the nervous system. In some cases the lesions of the nerve cells were more severe not on the injured but on the contralateral side. These and other facts of a similar character, and also the analysis of the qualitative and quantitative forms and variants of development of dystrophic changes within the nervous system, demonstrated the necessity of taking into consideration here not only the direct action of the pathogenic stimuli, but also the action of pathogenic stimuli operating through the nervous system.

Because the special features of the development and localization of dystrophic changes in the nervous system may shed light on certain aspects of the pathogenesis of many diseases and on the forms of the compensatory mechanisms of a protective and adaptive character brought into play under those circumstances, many authorities have sought suitable approaches to the investigation of these processes, paying great attention to the technique of their detection and study.



Changes in the vital staining power of the spinal cord (a), the brain stem (b), the cerebellum (c) and the cerebral hemispheres (d) of white rats after trauma to the left sciatic nerve. I) Content of dye in the tissues 1 hr after injection (sorption); II) Content of dye in the tissues 2.5 hr after injection (residue); III) ratio of sorption to residue (trophic potential).

From our previous work [1, 4] devoted to the investigation of the state of muscle tissue after trauma to the sciatic nerve, as shown by vital staining, we came to the conclusion that a close connection exists between processes of paranecrosis and processes of a neurodystrophic character. The phenomena of paranecrosis, expressing changes in the physico-chemical state of the protein substrate of the tissues in response to the action of the most widely differing stimuli in the environment, also reflect those changes in the tissues which are the result of the developing neurodystrophic process, underlying which are metabolic changes of varying severity.

In the present work we set out to use the method of vital staining to detect physico-chemical changes in the various divisions of the nervous system in connection with the development of dystrophic changes.

METHOD

The investigations were carried out on white rats weighing 130-150 g. In order to study the development of dystrophic changes within the nervous system, in these animals the sciatic nerve was divided and its central end was fixed with 0.02-0.05 ml of a 2% formalin solution. After 3, 7, and 14 days the animals were given an intra-

Changes in the Values of the Ratio of the Content of Neutral Red in the Tissues of the Left Side to its Content in the Tissues of the Right Side as a Result of Trauma to the Left Sciatic Nerve

Divisions of the central nervous system	Normal		3rd day		7th day		14th day	
	a*	b	a	b	a	b	a	b
Cerebral hemispheres	1	1	0,97	0,67	0,91	0,73	0,88	0,73
Crebellum	1	1,02	2,92	1,18	0,48	0,4	0,83	0,76
Brain stem	1,01	1,03	1,07	1,77	0,68	1,37	1,05	1,79
Spinal cord	1,0	1,08	2,06	2,06	1,36	1,48	1,11	3,95

* a) Ratio of dye content in tissues of left side to dye content in tissues of right side according to accumulation indices; b) ratio of dye content in tissues of left side to dye content in tissues of right side according to dye residue indices.

venous injection (into the saphenous vein) of a 1% solution of neutral red in Ringer's solution (without $NaHCO_3$) in a dose of 0.1 ml/30 g body weight, i.e., of 0.033 mg/g body weight of the dry stain. So that the degree of vital staining of the various divisions of the central nervous system (spinal cord, brain stem with the mesencephalon and diencephalon, cerebellum and cerebral hemispheres) could be estimated, on these days the animals were decapitated one hour after injection of the dye (in order to ascertain its degree of fixation) and 2.5 hours after injection (in order to ascertain the degree of excretion of the dye). A 1% solution of HCl in 96° ethyl alcohol was used for elution of the dye.

All the nerve formations were divided by a longitudinal incision into right and left halves. Weighed samples were taken and placed in the above solution for 24 hours, after which the quantity of vital dye was determined by colorimetry (with the FEK-M colorimeter). The dye content was calculated per gram body weight and expressed in μg. The results obtained were compared with those obtained in control animals.

The values of the fixation and excretion of the dye and the ratio between these values were used as indices of the degree of change in the nerve formations.

By this approach it is possible to determine not only the degree of damage to each particular nerve formation, but also their capacity to restore their original condition. These indices thus make it possible to ascertain the trophic state of the tissue, which may be defined quantitatively by the ratio between the values of the excretion and accumulation of the dye, expressed as a percentage. We also took into account the coefficients of asymmetry, i.e., the ratios between the values of accumulation and excretion of dye by the tissues of the injured side and the corresponding indices on the contralateral side. This coefficient enabled us to determine the degree of variation in the staining of the tissues of anatomically symmetrical areas at different levels of the central nervous system.

The values of accumulation and excretion of neutral red by the tissues of the control animals were characterized by insignificant individual variations (within limits of a few micrograms). An even smaller difference was observed between these values in the symmetrical areas of different divisions of the central nervous system of the same animal. In the experimental animals we took into consideration only those differences which were outside the limits of the variations found in the control animals.

RESULTS

In 15 operated animals (5 for each period) we studied the index of fixation of the vital dye, and in another 15 operated animals (also 5 for each period) – the index of excretion of the dye. Twenty rats (10 in each group) were used for determination of these indices in normal animals.

The results obtained are shown in the diagrams (see Figure, a, b, c, d). Analysis of the diagrams shows that along the path of development of the dystrophic changes in the central nervous system, significant changes were observed in the capacity to accumulate and to excrete the vital dye. The progress of the neurodystrophic process

was characterized above all by asymmetry in the accumulation and excretion of the dye, and hence by asymmetry in the trophic potential of the divisions of the central nervous system that were investigated (by the term "trophic potential" is understood the capacity of the tissue to normalize its physico-chemical state, which may be expressed by the ratio between the value of the accumulation of dye and the value of its excretion). It may be seen from the diagrams that in every case there was asymmetry in the accumulation of excretion of dye. The degree of asymmetry and its trend (towards increase or decrease) were different on the injured and contralateral sides. Asymmetry of these indices could also be observed at different periods of development of the dystrophic process. The actual values of this asymmetry are given in the Table.

The obvious asymmetry in the indices of accumulation and excretion of the dye (in all the investigated divisions of the central nervous system) is evidence of a reflex mechanism in its production, and the magnitudes of the corresponding changes express the quantitative aspect of the modified reactive power of the substrate under study.

After the application of trauma to the nerve, asymmetry of the trophic potential was also observed in nearly all cases (the ratio of the accumulation of the dye to its residual content in the tissues), which indicates that relationships are being formed between the processes of alteration and compensatory, restorative processes along the path of spread of the dystrophic changes within the nervous system.

It may be seen from the diagram (see Figure, a) that in spite of considerable changes in the accumulation and excretion of the vital dye, at nearly all periods the spinal cord maintained its trophic potential on the side of injury at the same level, whereas on the contralateral side this index was obviously increased on the 14th day, to several times its normal value. The tendency of the nerve tissue of the spinal cord to rid itself rapidly of the unwanted dye suggests the bringing into play of protective, compensatory mechanisms.

The fact that the trophic potential was maintained at the same level, despite the changes in the absolute values of accumulation and excretion of the dye, demonstrates the plastic functions of the nervous system, permitting the initial functional state to be maintained notwithstanding the action of the pathogenic stimulus.

In the second diagram (see Figure, b) it may be seen that the trophic potential of the brain stem fell much lower on the side of injury than on the contralateral side, and that this was due mainly to disturbance of the function of excretion of the dye.

As regards the cerebellum (see Figure, c) the opposite behavior was found – the trophic potential fell sharply on the contralateral side but increased considerably on the side of injury, and fell below normal only on the 14th day after division and fixation of the left sciatic nerve. This increase in the potential took place as a result of both a diminution in accumulation of the dye and an increase in its excretion.

The study of the dynamics of the trophic potential of the cerebral hemispheres showed (see Figure, d) that on the contralateral side (in relation to the injury to the sciatic nerve) this integral index was relatively stable, whereas on the injured side it increased considerably. In other words, the cerebral hemispheres not only compensated for the developing pathological process until the 14th day after injury to the nerve, but also put into action additional protective functions of a plastic character. It must be pointed out that the initial level of the trophic potential of the divisions of the central nervous system described above in normal conditions is far higher than the level of the trophic potential of the spinal cord.

As shown by the values of the trophic potential of the individual divisions of the central nervous system, it is thus possible to estimate not only the movement of the dystrophic changes within the nervous system but also the briging into play of the accompanying processes of a compensatory or restorative character [1]. In our experiments these processes were followed until the 14th day after injury to the nerve, and in no case did trophic ulcers develop. Corresponding to clinical manifestations of compensatory mechanisms, the manifestation of these mechanisms could be observed in the various divisions of the nervous system, and, in particular, in the cerebral hemispheres. It is important to ascertain in the future how these indices change in the various stages of development of tissue dystrophies at the periphery.

The method which we have developed makes it possible to trace the relationships existing between the changes in the central nervous system and processes arising in the corresponding tissues and organs. Analysis of these correlated processes may be important for the understanding of the mechanisms of development of systemic pathological processes and individual nosological forms when pathegenic stimuli of differing nature act upon the body.

SUMMARY

Rat tissues were stained with neutral red by introducing the dye into the blood. Both the sorption indices, and the ability of the tissues to get rid of the stain were taken into consideration. The ratio of the sorption of the dye to excretion was also studied. This ratio was the "trophic potential" of the tissues, since it characterized the degree of their compensatory ability. During development of the neurodystrophic process (caused by division of the sciatic nerve and its treatment with 2% formalin solution) asymmetrical changes of the staining indices were observed in various portions of the central nervous system (spinal cord, cerebellum, brain stem, cerebral hemispheres). The asymmetrical development of paranecrotic reactions in the central nervous system points to their reflex origin. The results obtained show that the higher portions of the nervous system possess greater plasticity than the lower, as manifested by the maintenance of the initial level of the "trophic potential" and the ability to increase it notwithstanding the development of dystrophic processess in the tissues. An close connection is inferred to exist between the processes of parabiosis and paranecrosis and trophical disturbances of the nervous system.

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REACTIONS OF THE DECENTRALIZED AND DENERVATED (PRE- AND POSTGANGLIONIC) HUMAN PAROTID GLAND TO REFLEX STIMULATION, PILOCARPINE, PROSERINE AND ATROPINE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 51-56, November, 1960
Original article submitted October 3, 1959

During the investigation of the secretion of saliva in patients with lesions at various levels in the central and peripheral nervous systems, we discovered several types of reaction to cholinergic (cholinomimetic and cholinolytic) and anticholinesterase (proserine) agents, and also to unconditioned reflex stimulation.

METHOD

We used Krasnogorskii-Yushchenko salivary irrigating capsules. Reflex salivation was studied by means of the application of 30 ml of 0.5% citric acid (at the rate of 10 ml per minute). The pilocarpine secretion was elicited by the subcutaneous injection of 0.5 ml of 1% pilocarpine, and the proserine secretion by the injection of 0.5-1.2 ml of a 0.05-0.1% solution of proserine. Atropine was injected subcutaneously in doses of from 0.3 to 1.2 ml of a 0.1% solution.

RESULTS

1. Decentralization (cortical) of the parotid gland arises in hemiplegias of central origin. Since 1933 we have investigated about 2000 such cases. On the side of the paralysis there is an increase in the reflex, pilocarpine and proserine secretions as a result of the freeing of the bulbar center and humoral automatism from the restraining influence of the cerebral hemisphers* (Fig. 1).

On the side of the lesion, on the other hand, a fall in the reflex, pilocarpine and proserine secretion of saliva takes place. After decentralization of the gland, atropine preserves its cholinolytic function, blocking the reflex and humoral secretion of both glands. However, since on the side of the paralysis there is an increase in both humoral and reflex secretion, its asymmetry is preserved after atropine too, although atropine depresses to a slightly greater degree the secretion of the gland on the same side as the lesion (Table 1).

2. Bulbar, nuclear decentralization arises in the presence of lesions situated in the region of the pons and medulla, when there is depression or suppression of the function of the salivatory centers (over 100 such patients investigated), together with an increase in the pilocarpine and proserine secretion (humoral automatism). Atropine depresses the secretion of both glands but does not correct the asymmetry (Table 2).

Strictly speaking, in nuclear lesions it is not decentralization of the gland which occurs, but denervation, due to interruption of the peripheral neuron or, more correctly, the nucleus of this neuron. In the paralysis

* In contrast to man, after decortication of one hemisphere in animals there is no asymmetry of secretion to humoral stimuli (E. A. Asratyan [1], T. M. Mal'tseva, and I. K. Zhmakin [4]). This fact shows that in man the opposite hemisphere fulfils a restraining function in relation to the humoral secretion of the contralateral gland.

TABLE 1

Patient A., Age 52 yr. Right-Sided Hemiparesis after Thrombosis of the Left Middle Cerebral Artery

Date of investigation	Character of secretion	Volume of secretion (in ml)	
		on the same side as the lesion	on the side of the paralysis
1959			
11/V	Reflex secretion	7.0	13.9
12/VIII	Spontaneous secretion during 15 minutes	1.0	2.5
	Secretion to proserine (1.2 ml of 0.05% solution)	10.0	15.0
19/VIII	Secretion to injection of pilocarpine (0.5 ml of a 1% solution)	25.2	30.4
21/VIII	Secretion to injection of atropine (0.5 ml of a 0.1% solution) with pilocarpine (0.5 ml of a 1% solution)	6.0	8.2

TABLE 2

Patient G., Age 50 yr. Thrombosis of the Right Posterior Cerebellar Artery (Wallenberg-Zakharenlo Syndrome)

Date of investigation	Character of secretion	Volume of secretion (in ml)	
		on the same side as the lesion	on the opposite side
1959			
21/VII	Spontaneous secretion	0.0	0.0
21/VII	Reflex secretion	2.1	4.8
18/VIII	Secretion to proserine (1.5 ml of a 0.05% solution)	2.7	1.4
20/VIII	Secretion to pilocarpine (0.5 ml of a 1% solution)	8.2	5.6
26/VIII	Secretion to injection of atropine (0.5 ml of a 0.1% solution) with pilocarpine (0.5 ml of a 1% solution)	2.3	1.7

TABLE 3

Patient B., Age 31 yr. Trauma to the Base of the Skull, Fracture of the Pyramid on the Left Side. Postganglionic Denervation

Date of investigation	Character of secretion	Volume of secretion (in ml)	
		on the same side as the lesion	on the intact side
1947			
18/VI	Spontaneous secretion	0.0	0.0
	Reflex secretion	0.0	3.5
24/VI	Secretion to pilocarpine (0.5 ml of a 1% solution)	9.1	7.4
17/IX	Secreation to injection of atropine (1.0 ml of 0.1% solution) with pilocarpine (0.5 ml)	262.6	3.0
1948			
15/V	Secretion to injection of atropine (1.0 ml of a 0.1% solution)	236.6	2.0
29/VI	Secretion to injection of proserine (1 ml of a 1% solution)	1.5	4.2

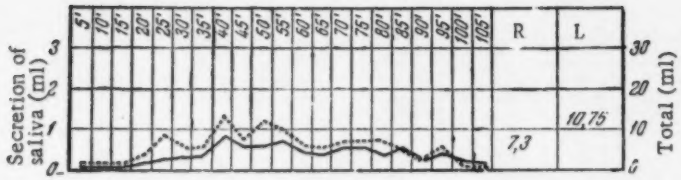

Fig. 1. Changes in secretion of saliva under the influence of injection of 0.5 c.c. of pilocarpine. ——— Secretion on the right; . . . . . secretion on the left. Patient Ol'kh-aya N. I.

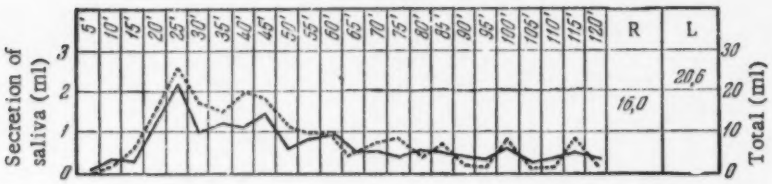

Fig. 2. Changes in the secretion of saliva under the influence of injection of 0.5 ml of 1% pilocarpine and 0.75 ml of proserine in a dilution of 1:1000. ——— Secretion on the right; . . . . . secretion on the left.

alternans of Millard-Gubler, Foville, Raymond-Cestan and Babinski-Nageotte (5 or more patients in each group were investigated), with lesions of the salivatory nuclei and their tracts, we have a combination of types 1 and 2, i.e., decentralization on the side of the paralysis and denervation on the side of the lesion. The pilocarpine secretion in these cases was increased on the side of denervation (Fig. 2).

3. Preganglionic denervation arises after interruption of the gustatory-secretory arc in an area beginning with the salvatory nucleus and the emerging roots of the glossopharyngeal nerve, then forming the tympanic plexus, and continuing further in the form of the nerve of Jacobson and the lesser superficial petrosal nerve to the otic ganglion. Denervation usually arises after a fracture of the base of the skull and of the pyramid of the temporal bone. Because of the interruption of the reflex arc, reflex secretion is absent or diminished, but later the humoral, pilocarpine secretion of saliva is increased. The proserine secretion is preserved as a result of the integrity of the postganglionic neuron. Atropine preserves its function of inhibitor of the secretory process, without affecting the character of the symmetry. Fourteen patients were investigated.

4. Postganglionic denervation also occurs after fracture of the base of the skull and of the pyramid of the temporal bone at the entry to the fallopian aqueduct, but in those cases in which, as a result of the extent of the trauma, the otic ganglion is also affected and the integrity of both the preganglionic and the postganglionic neuron is disturbed at the level of the otic ganglion – the secretory branch of the auriculotemporal nerve. Reflex salivation is suppressed, the secretion to pilocarpine is increased and that to proserine is diminished, and an atropine paradox develops, i.e., an extraordinarily intensive and prolonged secretion of saliva from the denervated gland in response to atropine (Table 3). Seven patients were tested.

We give below a scheme of the relationships appearing in all forms of denervation and decentralization (Table 4).

This scheme may be used for the differential diagnosis of the level of a lesion, especially with damage to the pre- and postganglionic neurons, for an indication of any distinctive clinical signs of these lesions is to be found in the literature.

In conclusion we may dwell on the role of proserine as an indicator in the determination of the level of denervation. Whereas pilocarpine reveals the presence of humoral automatism in all forms of decentralization and denervation, proserine reacts selectively depending on the integrity or disturbance of the pre- and postganglionic neurons, namely by producing an optimal secretory effect with a lesion of the preganglionic neuron and a less marked effect with a lesion of the postganglionic neuron. Proserine thus acts as an indicator of a disturbance

TABLE 4

Reaction Types of the Decentralized and Denervated Parotid Gland in Man

Serial No.	Types of reaction	Reflex secretion		Pilocarpine secretion		Proserine secretion		Reaction to atropine	
		on the side of the lesion	on the opposite side	on the side of the lesion	on the opposite side	on the side of the lesion	on the opposite side	on the side of the lesion	on the opposite side
1	Decentralization (cerebral hemispheres)	Decreased	Increased	Decreased	Increased	Decreased	Increased	Preserved	
2	Bulbar nuclear decentralization	Sharply decreased	Normal or decreased	Increased	Normal	Increased	Normal	Preserved	
3	Preganglionic denervation	Depressed	Normal	Increased	Normal	Uniform		Preserved	
4	Postganglionic denervation	Absent	Normal	Increased	Normal	Decreased	Increased	Atropine paradox	Preserved

Note. In decentralization (1) the opposite side is termed the side of paralysis.

Fig. 3. Changes in the secretion of saliva under the influence of injection of 0.5 ml of 1% pilocarpine. ——— intact gland; . . . . denervated gland. Patient Ves-ov P. K., age 52 yr.

of the most distal nerve formations, namely, the mechanisms of transmission from the nerve to the effector appratus. Pilocarpine-proserine dissociation is thus a characteristic sign of a disturbance of neuroglandular synaptic transmission. Pilocarpine-proserine dissociation is absent in decentralization and in nuclear and preganglionic denervation.

Postganglionic denervation is also accompanied by an atropine paradox.

Extreme degress of denervation of the parotid gland are thus characterized by suppression of proserine secretion and the presence of an extraordinarily intensive atropine secretion (proserine-atropine dissociation).

It must be considered that proserine has the presynaptic membrane (i.e.,the acetylcholine depot) as its point of application. Pilocarpine preserves its cholinomimetic properties in all forms of denervation. Its point of application is the receptive substance of the gland cells themselves. Since in normal conditions atropine blocks synaptic transmission but at the same time does not prevent the secretion of acetylcholine, i.e., does not block the presynaptic membrane, its point of application must be the postsynaptic structure. When this structure is destroyed, from being a paralyzer of saliva secretion, atropine becomes a stimulator. This part of the chemoreceptive substance of the postsynaptic membrane must be recognized, in contrast to a cholinopositive structure, reacting invariably to both pilocarpine and to atropine (after denervation) by a secretory effect, as a cholinonegative, cholinolytic structure (S. L. Levin [5]).

The development of this accessory cholinonegative structure in the process of evolution is the basis of the differential reaction to cholinergic antagonists.

It should be pointed out that G. Grundfest [2] concludes that the chemoreceptive postsynaptic structure consists of two parts – exciting and inhibiting.

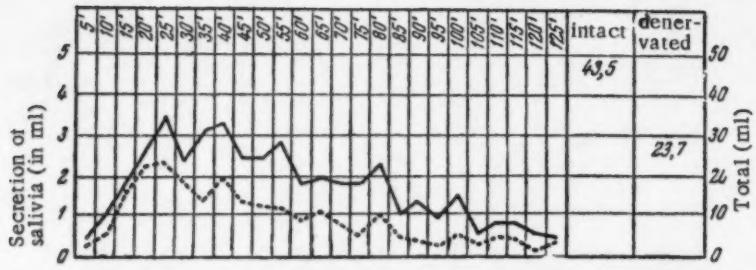

Fig. 4. Changes in the secretion of saliva under the influence of the simultaneous injection of 0.5 ml of 1% pilocarpine and 0.75 ml of 1% proserine. —— intact gland; denervated gland. Patient Ves-ov P. K., age 52 yr.

This supports our concept of the dual function of the postsynaptic formations.

We may thus consider from the foregoing remarks that the points of application of pharmacological agents in relation to the synaptic structures may be arranged in the following order: 1) proserine (presynaptic membrane), 2) atropine (postsynaptic structure) and 3) pilocarpine (acetylcholine) – the chemoreceptive part of the postsynaptic structure, in direct contact with the gland cells.

When the postganglionic neuron is intact, atropine, by interacting with the cholinonegative structure of the postsynaptic chemoreceptive system, produces a block of the secretory cells. When the cholinonegative structure is suppressed after postganglionic denervation, atropine comes into direct contact with the "exposed" cholinopositive (cholinomimetic) structure, producing a violent and extremely powerful cholinomimetic effect.

SUMMARY

The characteristic signs of decentralization (cortico-hemispheric and nucleo-bulbar) and denervation (pre- and postganglionic) of the human parotid gland are presented. These signs may be used for differential diagnosis. The significance of proserine and atropine as indicators of pre- and postganglionic denervation (proserine-atropine dissociation, atropine paradox) was demonstrated. The author illustrates the dual character of chemoreceptive postsynaptic transmission, namely, the presence of cholinopositive (cholinomimetic) phylogenetically older, and cholinonegative (cholinolytic, inhibitory) phylogenetically younger structures. Various special points of application of proserin, atropine, pilocarpine (acetylcholine) are given (within the range of the pre- and postsynaptic transmission).

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THE EFFECT OF ANALGESICS ON THE REFLEX REACTIONS OF THE CORONARY VESSELS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 57-61, November, 1960
Original article submitted November 28, 1959

One of the most important tasks of research on problems of the therapy of coronary insufficiency is to find pharmacological substances which can be used to influence the reflex reactions which bring about constriction of the cardiac vessels. These reactions are known to play a significant role in the development of angina pectoris attacks. In this connection, the mechanism of the effect of analgesic substances, which have been clinically observed to stop angina pectoris attacks, on the coronary circulation is an interesting question.

Our earlier investigations [2] showed that of the analgesic substances, morphine, thecodine [hydroxy-codeine hydrochloride], promedole [4-phenyl-4-propoxy-1,2,5-trimethyl-piperidine hydrochloride] and phenadone [Methadon], morphine alone has the property of dilating the cardiac vessels; the other analgesics, on the contrary, somewhat increase the tonus of the coronary vessels. One can therefore assume that the clinical efficacy of these agents is not due to their direct effect on the cardiac vessels.

The purpose of this investigation was to study the effect of analgesic substances on the reflex reactions of the coronary vessels in response to stimulation of the carotid sinus receptors and the afferent nerves.

There have been a series of investigations concerned with the question of how analgesic substances affect autonomic reflexes, but the results of these investigations are extremely inconsistent.

For example, Vercauteren [8], who investigated the effect of morphine on the reflex changes of the blood pressure in response to stimulation of the carotid sinus receptors, concluded that morphine, even in small doses, can inhibit these reflexes. According to Z. N. Ivanova's observations [1], promedole inhibits the cardiovascular and respiratory reactions elicited by stimulation of the lower respiratory tract. M. Yu. Ladinskaya [5] also observed analgesics to induce inhibition of the blood pressure reflexes which develop with pressure on a coronary artery.

Other researchers who studied the effect of analgesics on vascular reflexes, however, have concluded the exact opposite. For example, Vandenlinden [7] observed intensification of the blood pressure reflexes in response to stimulation of the carotid sinus receptors to occur under the influence of morphine. R. P. Kruglikova-L'vova [4] concluded that morphine and promedole increase the reflexes on the blood pressure induced by stimulation of the bladder interoceptors. G. V. Kovalev [3] reached a similar conclusion after studying the effect of analgesics on vascular reactions induced by stimulation of interoceptors.

From the brief review of the literary data given above, it is evident that the research which has been carried out on the effect of analgesic substances on autonomic reflexes is not systematic in nature and has obtained only contradictory results. No specific investigation has been made of the effect of these agents on the reflex reactions of the coronary vessels. This is, however, a question of great clinical, as well as theoretical, importance.

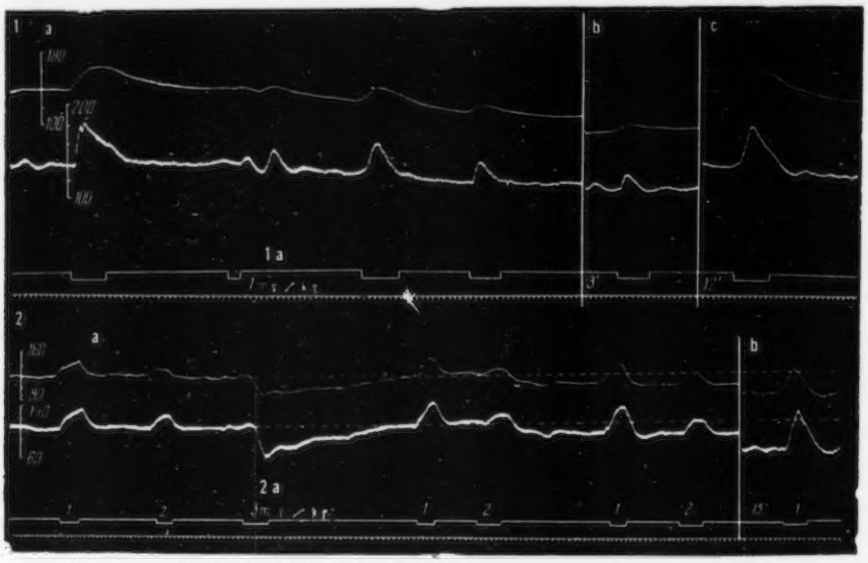

Fig. 1. Effect of morphine (1 mg/kg) on the reflex reactions of the coronary vessels: a) inhibition of reflexes from the tibial nerve affecting the coronary vessels and blood pressure caused by morphine in a dose of 1 mg/kg; b) increase in reflexes from the tibial nerve (1) and the carotid sinus (2) affecting the coronary vessels caused by morphine in a dose of 3 mg/kg. Curves show (from top to bottom): resistogram; blood pressure; stimulation of reflexogenic zones and administration of morphine; time (in 5-sec marks). Key: 1a) Morphine; 2a) morphine.

METHODS

The experiments were conducted on cats anesthetized with arethan and chloralose. The method of resistography was used to determine the effect of pharmacological substances on the reflex reactions of the heart. This method, proposed by V. M. Khayutin [6], is based on the principle of artificial stabilization of the blood flow in the vessels and uses a special pump to perfuse the vessels with the animals' own blood. A detailed description of this method and a diagram showing the experimetal plan can be found in an earlier article of ours [2].

A catheter was introduced into the mouth of the left coronary or circumflex artery; blood from the carotid artery entered this catheter in a volume which was constant per unit of time. Under these conditions, the pressure recorded at the pump outlet reflects change in vascular resistance, rising with constriction and falling with dilatation of the vessels. The advantage of this method is that it makes it possible to estimate changes in the tonus of the cardiac vessels independently of the changes in systemic arterial pressure which occur in the organism. In connection with the study of the reflex reactions of the coronary vessels, this is of particular value, because the reflex changes in the blood pressure developing in response to stimulation of the afferent system can mask the true condition of the tonus of these vessels when the estimations are based on measurement of the blood flow in the vessels of the heart.

The blood pressure was recorded in the femoral artery with a mercury manometer. Heparin (800-1000 units/kg intravenously) was used to prevent blood coagulation.

Reflexes on the cardiac vessels were induced by stimulating the carotid sinus receptors (pressure on the carotid artery) and the afferent fibers of the tibial and medial nerves. This stimulation was effected by square-wave pulses with a frequency of 50-60 hertz and a voltage of 5-15 volts.

RESULTS

The experiments conducted demonstrated that analgestics, in relatively small doses, can inhibit the reflexes of the cardiac vessels. For example, morphine in doses of 1-2 mg/kg decreased the reflexes an average of

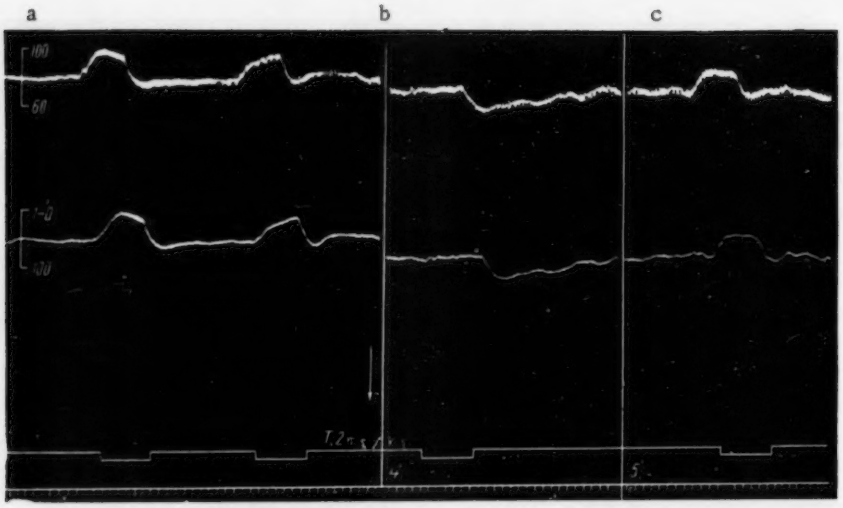

Fig. 2. Effect of thecodine on the reflex changes in the resistance of the coronary vessels and in the blood pressure provoked by stimulation of the carotid sinus. Symbols the same as in Fig. 1.

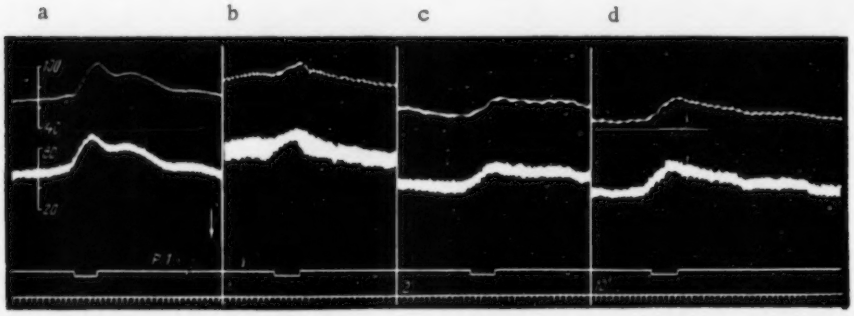

Fig. 3. Effect of phenadone on the reflex changes in the resistance of the coronary vessels and in the blood pressure provoked by stimulation of the medial nerve. Symbols the same as in Fig. 1.

50-70% of the original values. Their complete inhibition was even observed in a few cases. The effect of the morphine was not lasting. The reflexes usually returned to the original level 12-15 minutes after the administration of the preparation (Fig. 1a). Increasing the morphine dose to 2.5-3 mg/kg did not enhance the effect. In a series of experiments in which morphine was administered in doses in 3-4 mg/kg, moreover, the reflexes of the coronary vessels increased 5-10% of the original level. The reflexes remained increased for some time (1½-2 hours). In most cases, we were unable to observe the restoration of the reflexes to the original level due to the conditions of the method employed (Fig. 1b).

Thecodine had a similar effect on the reflex reactions of the coronary vessels. It should be noted, however, that thecodine's effect on these reflexes was more pronounced than that of morphine. Even in doses of 0.3-0.5 mg/kg, thecodine inhibited the reflexes 30-40% of the original level. When the dose was increased to 1-2 mg/kg, the resulting inhibition of the reflexes usually amounted to 60-80% (Fig. 2). Thecodine did not cause lasting inhibition of the reflexes; the original reflexes were usually restored after 12-15 minutes. Administered in large doses (3-3.5 mg/kg), thecodine, like morphine, caused the reflexes of the cardiac vessels to increase. In may experiments, this increase was very pronounced – as much as 80-90% of the original values.

Promedole also showed an ability to inhibit the reflexes of the cardiac vessels. In doses of 0.3-0.5 mg/kg, it caused the reflexes to decrease 75-85% of their original level. The inhibitory effect of promedole on the reflex

reactions of the coronary vessels was most clearly expressed when the drug was administered in small doses. When the promedole dose was increased to 2-3 mg/kg, the reflexes either did not change or, sometimes, increased slightly (4-5% of the original).

Phenadone, like the other analgesic substances, caused inhibition of the reflex reactions of the coronary vessels. When administered in doses of 0.5-1 mg/kg, it inhibited the reflexes by 30-60% of the original (Fig. 3). Under our experimental conditions, it was inconvenient to use phenadone in larger doses, because, in doses such as 2-3 mg/kg, phenadone induces disturbances of cardiac activity and acute blood pressure fall.

All the experimental analgesic substances, therefore, in relatively small doses, cause inhibition of the reflex reactions of the coronary vessels. These doses of the analgesics, however, roughly correspond to the therapeutic. The use of these substances in larger doses, however, caused the reflex reactions of the cardiac vessels to increase in a majority of cases. The different effects caused by the different doses of the analgesic substances are, it would seem, one explanation for the contradictory conclusions reached by the various researchers concerned with this question.

It should also be noted that the degree of change induced by the analgesics in the reflexes of the coronary vessels was identical regardless of which reflexogenic zone was stimulated to produce them.

The changes in the blood pressure reflexes, which were recorded at the same time as those of the cardiac vessels, were parallel to the changes in the reflexes affecting the coronary vessels.

Earlier investigations [2] allowed us to establish that morphine alone of the experimental analgesic substances (morphine, thecodine, promedole and phenadone) can increase the blood supply of the heart. Promedole and phenadone, in a majority of cases, increased the tonus of the coronary vessels, thus reducing the volumetric rate of the coronary blood flow. Thecodine caused no significant changes in the condition of the coronary circulation.

On the basis of these data, it would seem that the efficacy of most analgesic substances in angina pectoris is not due to their direct effect on the vessels of the heart. As the data of the present investigation have shown, however, analgesic agents can inhibit the reflex reactions causing constriction of the cardiac vessels when administered in doses corresponding to the therapeutic.

These data allow the hypothesis that the efficiency of analgesic substances in angina pectoris is due not only to their elimination of the pain syndrome, but also to their ability to inhibit the reflex reactions which cause constriction of the coronary vessels and therefore promote the development of coronary circulatory disturbances.

When using these analgesic substances clinically, one should also keep in mind the fact that these substances, in larger doses, can promote the deterioration of myocardial blood supply conditions by raising the tonus of the coronary vessels or intensifying their reflex reactions.

The only exception to this rule is morphine, which, in a majority of cases, improves the myocardial blood supply.

SUMMARY

The author studied the effect of analgetic substances (morphine, thecodine, promedol, and phenadon) on the reflex reactions of coronary vessels, provoked by stimulating the carotid sinus receptors and afferent nerves. The analgetic substances given in therapeutic doses are capable of depressing the mentioned reflexes.

Conversely, in a number of cases large doses of the analgetic increased the reflex reactions of coronary vessels.

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THE EFFECT OF ARECOLINE ON THE CARDIAC ACTIVITY OF THE CHICK OF THE CHICK EMBRYO AT VARIOUS STAGES OF ITS DEVELOPMENT

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Translated from the Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp 61-65, November, 1960
Original article submitted August 17, 1959

The literature contains many papers concerned with the question of the effect of vagotropic substances on the heart of the chick embryo. Many authors, using various isolated-heart methods, have ascertained the periods of embryonal development at which the effect of these substances on cardiac activity is apparent and compared them with the presence or absence of nerve elements in the embryonal heart. The results obtained are somewhat inconsistent. Some authors observed the effect produced by the action of vagotropic substances during the pre-innervated stage of cardiac development [12, 13, 16, 18], while others believe that the effect is not apparent until nerve elements have appeared in the heart [11, 14, 17].

In our opinion, the study of this question should be based on the assumption that specific cholinergic structures which participate directly in the realization of the vagus effect are present in the myocardium [1] rather than on the fact of the development of the nerve elements.

Having taken this position, we decided to ascertain how arecoline affects the heart of the chick embryo at various stages of its development as a step towards understanding the problem of the development and manifestation of the inhibitory process, using as the model the cardiac activity of an intact embryo and taking measures for maximum viability of the latter.

METHODS

In the work, we recored the ECG of the chick embryo, providing for its maximum viability [5]. To this end, the egg containing the embryo was placed in a heated chamber; the egg and the embryonal membranes were opened, and the bio-electric potentials of the heart were led off by means of electrodes. After the background frequency of the embryonal heart beat was recorded, arecoline was injected into the thigh in a dose of 100 γ. The frequency of the heart beat was then recorded one, three, five and ten minutes after the arecoline injection. The embryo was then injected with 10γ atropine, after which the frequency of the cardiac contractions was again recorded three, five and ten minutes after the injection. The doses of arecoline and atropine used for each stage of embryonal development were the same to permit comparison of the results obtained. Seventy-three embryos at stages ranging from the 8th to 19th day of development were used in the experiments.

RESULTS

The data obtained showed that arecoline in the dose specified does not exert its characteristic effect on the cardiac activity of chick embryos at early stages of development. We observed no pronounced changes of any kind in the frequency of the cardiac contractions after the administration of the vagotropic substance on the

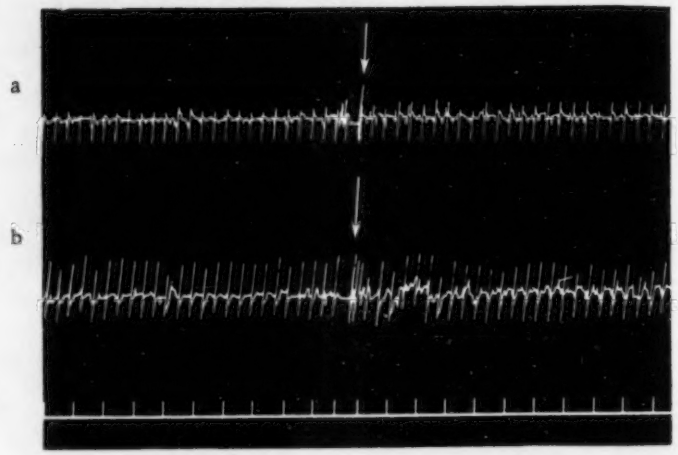

Fig. 1. Absence of change in the frequency of the cardiac contractions after the administration of arecoline to chick embryos on the eighth (a) and ninth day of development (b). Time shown in seconds; arrow represents the arecoline injection. Recorded one minute after the arecoline injection.

8th-9th days of development (Fig. 1). On the eighth day, the injection of atropine on a background of the action of arecoline had no apparent effect either. On the ninth day, a few cases showed some increase in the frequency of the heart beat after the atropine injection.

On the tenth day of development, we observed a more or less expressed decrease in the frequency of the heart beat in several cases the first and third minutes after the arecoline injection, averaging 10-20% of the original level. Five minutes after the arecoline injection, the frequency of the cardiac contractions had usually returned to the original background level.

A peculiar type of cardiac reaction, in the form of alternate decrease and increase in the frequency of the heart beat during the action of arecoline, was observed in a few embryos on the tenth day of development. The unstable, oscillating nature of these changes in the frequency of the cardiac contractions would seem to indicate that the tenth day of development is the first day when the effect of the action exerted by the vagotropic substance is apparent.

After the tenth day of development, this effect became stable, increasing in degree as the embryo grew older. On the 11th day, the decrease in the frequency of the cardiac contractions following the arecoline injection averaged 25-40% of the background frequency, and on the 15th-19th day, this decrease reached an average of 50-60% (Figs. 2 and 3).

The decrease in the frequency of the heart beat was often attended by arrhythmia. The effect of the arecoline injection was usually apparent by the end of the first and beginning of the second minute. Cardiac arrest occurred in a few embryos, but was dispelled by the atropine injection. A somewhat unusual occurrence, difficult to account for, was observed with the repeated administration of arecoline to the same embryo. Although the first injection caused the frequency of the heart beat to decrease, returning to normal by the end of the tenth minute, subsequent injections of arecoline had no effect, even when the substance was injected in a dose of 500 γ.

Atropine, administered to the chick embryos during the decrease in the frequency of the heart beat caused by the arecoline injection, restored the heart rate to its original level; often, we even observed some increase in the frequency of the heart beat over the background level. The atropine injection usually dispelled any arrhythmia which had developed after the arecoline injection.

Visual observation of the embryo's behavior showed a considerable increase in the motor activity of the embryo after the arecoline injection. The movements were irregular and disorganized in nature, alternating with

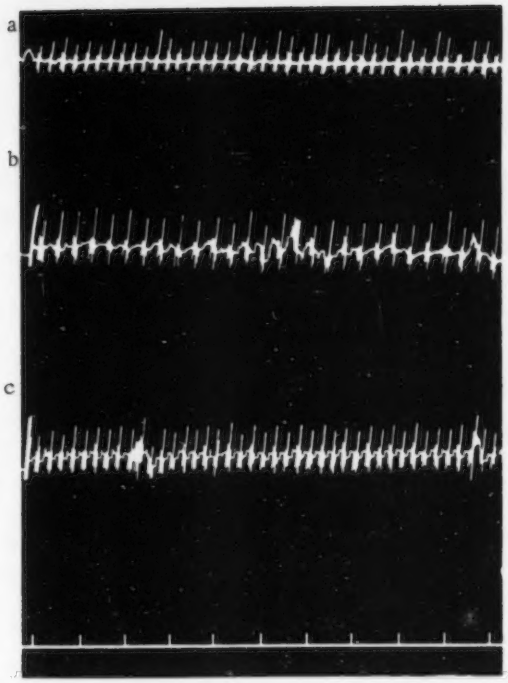

Fig. 2. Change in the frequency of the cardiac contractions effected by arecoline in a chick embryo on the 13th day of development. a) Background frequency of heart beat; b) decrease in frequency of heart beat one minute after arecoline injection; c) restoration of frequency of heart beat three minutes minutes after atropine injection. Time shown in seconds.

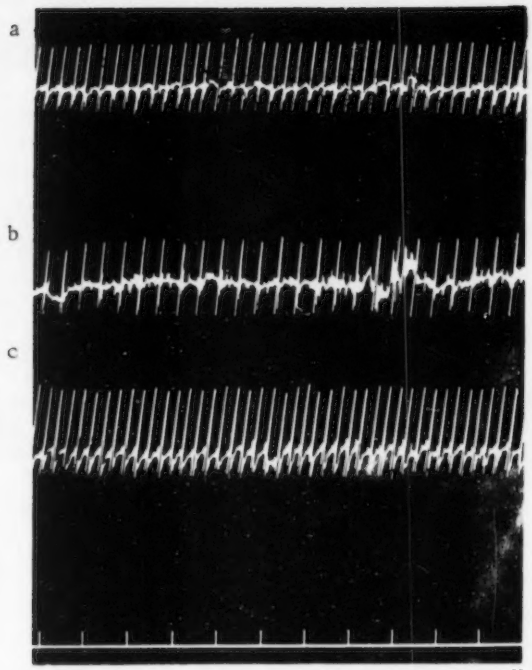

Fig. 3. Change in the frequency of the cardiac contractions effected by arecoline in a chick embryo on the 19th day of development. a) Background frequency of heart beat; b) decrease in heart beat frequency one minute after arecoline injection; c) restoration of original heart beat frequency three minutes after administration of atropine. Time shown in seconds.

short resting periods. Stimulation of the embryo's respiratory movements was observed in several cases after the injection of arecoline on the 19th day.

Several variants of control experiments were conducted in order to establish the exact focus of the action of of arecoline: 1) injection of distilled water instead of arecoline (which caused no change in the rate of the heart beat); 2) injection of arecoline on a background of the action of atropine (arecoline did not exert its characteristic effect on cardiac activity in these experiments). Since atropine, which blocks cholinergic systems, prevented the manifestation of the effect of arecoline, the control experiments confirmed the fact that arecoline is a vagotropic substance and acts on specific cholinergic systems which directly participate in the realization of the inhibitory reaction of the heart. On this basis, the manifestation of arecoline's effect, and therefore, the manifestation of the inhibitory effect of the heart, must be associated with maturation of the analogous physiologic mechanisms or cholinergic structures.

The data obtained show that the cholinergic structures are not functionally in evidence during the initial period of embryogenesis (8th-9th day). Their functional manifestation begins at a definite stage of embryonal development (10th day) but is not immediately complete. In many cases, the inhibitory reaction of the heart was not yet in evidence on the tenth day of development, although it was already clearly apparent in other cases. The oscillating nature of the reaction to arecoline observed in some embryos – periods of decreased heart beat frequency alternating with periods of increased frequency – indicated incomplete functional maturity of the structures producing inhibition of cardiac activity. On subsequent days of embryonal development, the inhibitory reaction of the heart was clearly apparent and stable, increasing with the development of the embryo. It was

interesting to observe this characteristic development of cardiac inhibition, which showed the secondary nature of the formation of the inhibitory reaction and the division between the development and manifestation of stimulating and inhibitory influences.

Adrenalin, which stimulates excitation in the substrate, manifests its effect on the heart at the stage of its development observed during the primitive epithelial tube stage [7], but, as our experiments have shown, the capacity for inhibitory reaction is developed much later. Some of the literary data also testify to the later manifestation of the inhibitory reaction of the embryonal heart [2, 5].

This hypothesis is further corroberated in the work of N. A. Itina [6]; after conducting a phylogenic and ontogenic study of the properties of the muscls of various organs, this author concluded that the inhibitory effect of the nervous system on the heart has, in the course of the phylogenic animal development become a secondary phenomenon.

The theory that there is some division between the inhibitory and stimulating processes has been expressed before by I. P. Pavlov (1934). This theory was then developed by L. A. Orbeli [8] and D. A. Biryukov [3], who asserted that the nature of the evolutionary development of nervous activity has ended in the gradual and separate formation of the inhibitory function.

In discussing the question of the division between the development and manifestation of the inhibitory reaction, we have no wish to analogize the inhibitory processes in the central nervous system and in the heart. But posing the question of the times of development of the inhibitory mechanism in the myocardium is commensurate, since excitation and inhibition are functions not only of the central nervous system, but also of highly specialized structures in general [3].

The inhibition effect was first discovered on the model of cardiac activity (Weber, 1845), and discovery of the sequence of development of excitation and inhibition in the heart during embryogenesis can help advance our knowledge of how the interrelations of the principal nervous processes are formed.

SUMMARY

Arecolin which acts on the choline-reactive systems and simulates vagal effect, does not exert typical action on the cardiac activity of chick's embryo 8-9 days old. The tenth day is the first in which arecolin influences cardiac rate. Beginning with the 11th day, administration of arecoline produces constant effect, the magnitude of which grows with the age of the embryo.

Based on the above data the author concludes that the inhibitory reaction of the heart appears and manifests itself at a much later date, and is formed gradually.

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THE USE OF AN EXPERIMENTAL MODEL OF HYPERTONIA FOR ASSESSING THE EFFICIENCY OF SCHIZANDRA CHINENSIS IN ATHEROSCLEROSIS

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Translated from the Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp 66-70, November 1960
Original article submitted January 18, 1960

The experimental cholesterol model of atherosclerosis has been successfully used in research studying the significance of certain basic pathogenic factors of this disease, as well as to test many recently proposed methods of the prophylaxis and therapy of atherosclerosis [1]. Experimental research has made possible the clinical use of many pharmacological substances, vitamins, hormones, etc. to prevent and treat atherosclerosis [9]. In the case of patients suffering from hypertonic disease along with atherosclerosis, however, many of these agents have proved to be rather ineffective.

These two conditions are known to occur frequently in combination. It has been observed both clinically [8, 10] and experimentally [5, 13] that arterial hypertonia is one of the factors intensifying the development of atherosclerosis. Since many atherosclerosis patients suffer simultaneously from hypertonia, further research is needed to find new agents to prevent and treat atherosclerosis, and such agents should be tested on an experimental model of hypertonia combined with atherosclerosis, instead of on an experimental model of atherosclerosis alone, as has been done up to now.

The purpose of our investigations was to make a comparative study of the efficiency of Schizandra chinensis seeds on both an experimental model of atherosclerosis and a model of hypertonia combined with atherosclerosis.

There are no literary data concerning the influence of Schizardra chinensis on the development of experimental atherosclerosis.

METHODS

The investigations were performed on 28 mole Chinchilla rabbits weighing 2.4-3 kg each, which had been kept under identical conditions and fed the customary diet. The animals were divided into two equal groups of 14 rabbits each.

Before the experiments with experimental atherosclerosis were begun, the common carotid artery in eight of the rabbits was exposed through a skin flap. When the post-operative wound had healed, systematic measurements of the rabbits' blood pressure were made until the normal blood pressure values were established. Then the main trunks of the right and left renal arteries were constricted by ligation with a wire 0.7-0.8 mm in diameter in order to reproduce hypertonia.

In all the rabbits before the experiments began, we determined the total blood cholesterol content according to M. A. Levchenko and the lecithin content according to Samson's method; after the experiments began, the

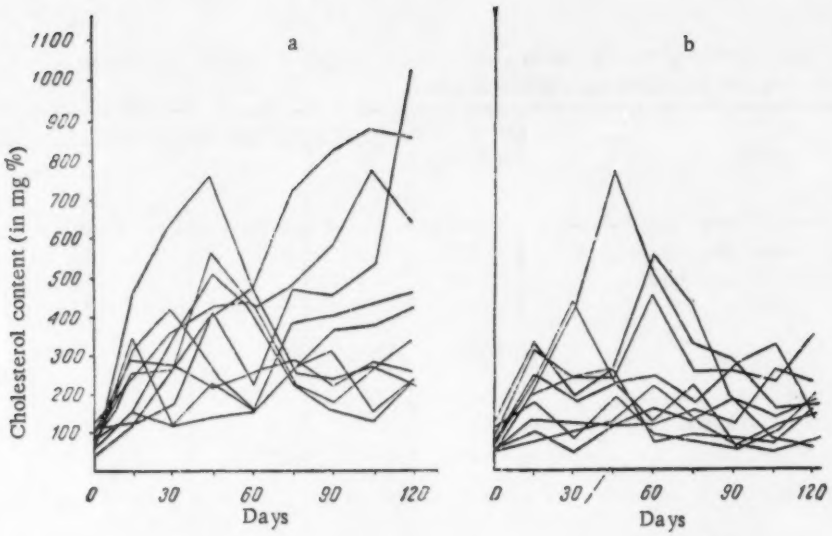

Fig. 1. Effect of Schizandra chinensis on the blood cholesterol content of the control (a) and experimental (b) rabbits with experimental atherosclerosis.

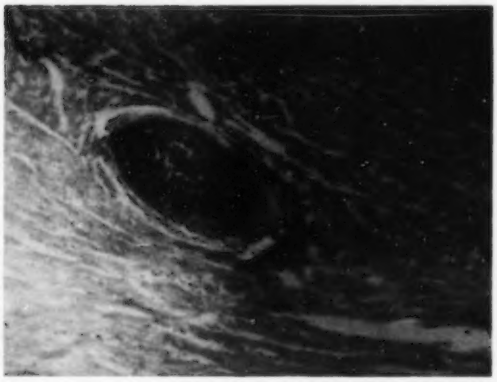

Fig. 2. Acture lipoidosis of an intramuscular artery of the heart in a rabbit given cholesterol. Stained with Sudan III and hematoxylin. Photomicrograph 35x.

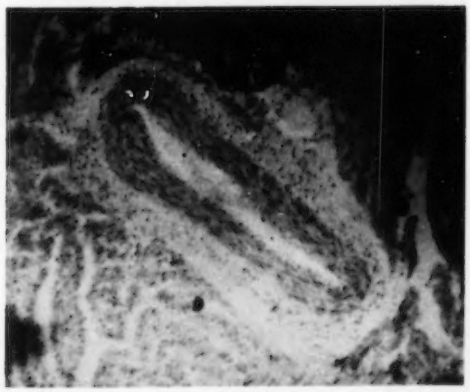

Fig. 3. Absence of lipodosis in myocardial artery of rabbit given cholesterol plus Schizandra chinensis. Stained with Sudan III and hematoxylin after-stain. Photomicrograph 35x.

biochemical examinations of the blood were carried out every 15-17 days.

Experimental atherosclerosis was induced in the control group of rabbits by the daily administration of 0.2 g/kg cholesterol in a 10% sunflower oil solution, introduced into the stomach with the aid of a probe.

The other group of rabbits received the same daily dose of cholesterol plus a 0.2 g/kg dose of ground Schizandra chinensis seeds.

At the end of the experimental period (four months), the rabbits were sacrificed by air embolism; the aorta and heart were extracted and fixed in 20% formalin.

The aorta was stained totally with Sudan III, and the changes observed in it were diagrammed schematically.

TABLE 1

Degree of Atherosclerotic Changes in the Aorta and Coronary Arteries of Normotonic Rabbits Killed after Four Months of Experiments with Experimental Atherosclerosis

Rabbit group	Number of rabbits	Degree of atherosclerosis				No changes
		++++	+++	++	+	
Aorta						
Control (received cholesterol alone)	10	2	3	4	1	–
Experimental (received cholesterol + Schizandra chinensis)	10	–	1	3	2	4
Coronary arteries						
Control (received cholesterol alone)	10	5	1	1	1	2
Experimental (received cholesterol + Schizandra chinensis)	10	2	–	3	1	4

Key: +, slight atherosclerotic changes; ++, moderate changes; +++, acute changes; ++++, very acute changes.

The degree of atherosclerotic changes was also studied mircoscopically on a series of heart sections, stained for fat with Sudan III.

RESULTS

The total blood cholesterol content in all twenty normotonic rabbits, i.e., those without partial constriction of the renal arteries, ranged between 55 and 100 mg% before the experiments began. Two weeks after the start of cholesterol feeding, the blood cholesterol content had increased to 350-450 mg% in the majority of the control rabbits (Fig. 1).

As Fig. 1 shows (left-hand curve), the blood cholesterol level was still higher on the 45th day of the experiments; in six out of ten rabbits, it was as high as 400-778 mg%. Thereafter, the blood cholesterol content remained high in the majority of rabbits until the very end of the experiments. By the end of the fourth month, of cholesterol feeding, for example, it was 638-1025 mg% in three rabbits.

The animals which were given Schizandra chinensis as well as cholesterol showed a relatively low cholesterol level (see Fig. 1b), unlike the control rabbits. In most of the experimental rabbits, the cholesterol level usually fluctuated between 50 and 250 mg%. Relatively high indices (400-770 mg%) were only observed at certain times in four out of ten rabbits. By the 90th-120th day of the experiments, however, the cholesterol level had decreased to 64-225 mg% (in nine out of ten rabbits).

It should be noted that the most acute vascular atherosclerotic changes were observed in the rabbits with a high blood cholesterol content.

Aortic lipoidosis was especially pronounced in the animals of the control group (Table 1).

As Table 1 shows, acute atherosclerotic changes were found in five out of the ten control rabbits; in these animals, the aortic walls were swollen, and the internal surface of the aorta was covered with large atherosclerotic plaques merging into each other. Moderate atherosclerotic changes of the aorta were found in four out of the remaining five rabbits in this group, and slight changes were observed in one rabbit.

Acute changes were observed in the myocardial vessels of all calibers in five of the ten control rabbits. Plaques constricting the arterila lumen were found in the intramuscular arteries and main subepicardial trunks of the coronary vessels. Cholesterol crystals were apparent among the lipoid deposits. The lumen of many of the intramuscular arteries was closed (Fig. 2).

Figure 2 shows acute lipoidosis of an intramuscular artery of the heart with closure of its lumen. In the control group, the coronary arteries of only two rabbits showed no change.

In the rabbits which were given Schizandra chinensis, the aorta showed a completely different picture.

TABLE 2. Cholesterol and Lecithin Content in the Blood and Degree of Atherosclerosis of the Aorta and Coronary Arteries in Hypertonic Rabbits on the 120th Day of Systematic Administration of Cholesterol and Cholesterol Plus Schizandra Chinensis

Rabbit group	Rabbit No.	Total blood cholesterol (in mg %)	Blood lecithin (in mg %)	Lecithin cholesterol coefficient	Degree of atherosclerosis	
					of aorta	of coronary vessels
Control (given cholesterol)	21	1 157	825	0,7	++++	+++
	22	1 182	816	0,72	++++	+++
	23	1 401	824	0,58	+++	++
	24	550	382	0,69	+++	++++
Experimental (given cholesterol + Schizandra chinensis)	25	416	378	0,9	++++	+++
	26	750	535	0,7	++++	++
	27	1 120	459	0,4	++	+++
	28	551	361	0,65	++	++++

Key the same as in Table 1.

No atherosclerotic changes were found at all in the aorta of four out of ten rabbits, in two rabbits, only tiny lipoid spots were seen, moderate changes were observed in three animals, and acute lipoidosis was discovered in the last rabbit.

In the coronary arteries of only two out of ten rabbits could we observe expressed lipoidosis of the intramuscular arteries. Moderate atherosclerotic changes of the coronary arteries were found in three rabbits and slight changes, in one. Both the main trunks of the coronary arteries and the intramuscular vessels of the other four rabbits were completely unaffected. As an example, we include a photomicrograph of a heart section taken from rabbit No. 18, showing one of the intramuscular arteries, free of lipoidosis (Fig. 3).

Since the administration of Schizandra chinensis inhibited or prevented the development of atherosclerosis in the normotonic rabbits, it was interesting to study the effect of this agent on the development of experimental atherosclerosis in the hypertonic rabbits. For this purpose, we conducted experiments on the eight hypertonic rabbits (four in each group) at the same time as the experiments on the normotonic rabbits.

In the hypertonic rabbits, the maximal arterila pressure was 80-120 mm of mercury before the operation, increasing after the operation to 175 mm. A gradual decrease of the arterial pressure to 130 mm of mercury was observed at the beginning of the fourth month of the experiments; the curve of arterial pressure decrease was steeper in the rabbits given Schizandra chinensis.

In the hypertonic rabbits, unlike the normotonic animals, the total blood cholesterol content was high from the very first weeks of the experiment, reaching even higher figures towards the end of the fourth month (Table 2).

Table 2 shows a high cholesterol content and sharply reduced lecithin/cholesterol coefficient in both groups of hypertonic rabbits.

The atherosclerotic changes found in the aorta and coronary arteries of both the control hypertonic rabbits and those which were given Schizandra chinensis were more pronounced than the changes found in the normotonic group of rabbits.

Therefore, the data obtained show that cholesterol feeding caused the same serious atherosclerotic changes in the hypertonic rabbits given Schizandra chinensis as it did in the hypertonic rabbits which received only cholesterol.

The inhibition or complete prevention of the development of atherosclerosis observed in the normotonic rabbits which received Schizandra chinensis was evidently due primarily to the stimulating and tonicizing action of the drug on the central nervous system [4, 7], secondly to an increase in the activity of certain enzymes,

intensification of the carbohydrate [2] and phosphorus [3], metabolisms and stimulation of internal respiration [6] and thirdly, to the fact that the introduction into the organism of Schizandra chinensis causes an increase in the systolic volume of the heart [11], while an infusion of the leaves intensifies the minute volume of a weakened, hypodynamic heart [12].

The positive influence of Schizandra chinensis is not manifested, however, under conditions of hypertonia, a powerful pathologic factor.

SUMMARY

Experiments with 28 rabbits have shown that seeds of Schizandra chinensis administered in a dose of 0.2 g/kg for experimentally induced atherosclerosis will reduce the cholesterol level in the blood and decrease or totally prevent atherosclerosis of the aorta and of the coronaries in the normotonic rabbits. The same doses of S. chinensis administered to hypertensive rabbits fail to exert any effect. In the search for effective agents for prophylaxis and treatment of atherosclerosis it is necessary to test them not only on models of experimental atherosclerosis, but likewise on models of atherosclerosis associated with hypertension.

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THE IMMUNOLOGICAL ACTIVITY OF ANTIBODIES, ADSORBED ON SOLID ADSORBENTS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 71-76, November, 1960
Original article submitted November 20, 1959

The properties of antibodies when adsorbed on solid adsorbents have not been adequately studied. In the accessible literature we found only isolated reports of this subject. Eisler [3], for example, showed that diphtheria and tetanus antitoxins, when adsorbed on activated carbon, lose their power to neutralize homologous toxins. According to Vekerdi [4], precipitins against horse proteins, when adsorbed on animal charcoal, talc, powdered glass, starch, erythrocytes, barium chloride, agar agar and barium sulfate, also showed no immunological activity when mixed with the homologous antigens.

In the search for methods of improving serological reactions, we studied the properties of antibodies adsorbed on solid adsorbents.

METHOD

In view of the negative results obtained by Eisler [3] and Vekerdi [4] with adsorbed antitoxins and precipitins, we carried out experiments with different antibodies and antigens. Altogether 112 experiments were conducted with 40 strains of various species of microorganisms, 2 strains of influenza viruses, complete antigens of dysentery bacteria and of the genus Salmonella, and also with haptens and type A botulinus toxin.

The antibodies were adsorbed by the following method. To 2 ml of an immune serum,* diluted 1 : 10 with physiological saline, was added 100 mg of the adsorbents to be tested, in powder form and previously sifted through a standard brass grating of 40 μ mesh (for standardization of the particle size). The sera with the adsorbents were kept for 2 hours at 37° and for 18 hours at 4-8°, and periodically stirred with a glass rod. The sera with adsorbents were then centrifuged at 2000 rpm. The translucent supernatant fluid was removed by decantation and the residue was suspended in 10 ml of physiological saline, containing 1% boric acid as preservative. The suspensions of adsorbents thus obtained contained on their surfaces particles of antibody adsorbed from the immune sera.

The study of the immunological activity of antibodies adsorbed on solid adsorbents, by various methods, has shown that the simplest and most convenient method is the agglomeration reaction on a glass slide. If a suspension of an adsorbent, containing adsorbed antibodies on its surface, is mixed with homologous antigen on a glass slide, the particles of adsorbent merge to form large agglomerates. This does not occur if adsorbents, containing adsorbed antibodies on their surface, are mixed with heterologous antigens. To perform the agglomeration reaction, on to a thoroughly degreased glass slide were placed three drops of the suspension for testing, on the particles of which the antibodies were adsorbed. To the first drop of suspension was added a drop of homologous antigen, to the second drop – a drop of heterologous antigen, and to the third drop – a drop of physiological saline. After

* Sera were used which were preserved in 1% boric acid. Sera which were preserved with other elements and sera which underwent lypophilic drying processes were not usable for adsorption experiments.

thorough mixing of each drop with a platinum loop, observations were made for a period of 5 minutes (with slight agitation of the slide) of the changes in the structure of the suspension. The reaction was regarded as specific if agglomeration took place only in the drop in which the particles of adsorbent were mixed with homologous antigen, and in the other two drops the suspension remained homogeneous. The intensity of the agglomeration reaction was denoted by + signs.

As a result of trials of suspensions of various substances possessing the property of adsorbing antibodies (A. K. Adamov [1]), it was found that only suspensions of alizarin, infusorial earth and animal charcoal were suitable for the study of the immunological activity of adsorbed antibodies. Suspensions of these adsorbents, after adsorption of antibodies, remain stable and do not give nonspecific agglomeration with physiological saline or heterologous antigens. Comparison of the sensitivity of suspensions of animal charcoal, infusorial earth and alizarin, containing adsorbed antibodies on the surface of their particles, showed that a suspension of alizarin was most active, i.e., reacted with the smallest quantity of antigen. The activity of the antibodies adsorbed on the above-mentioned adsorbents depended not only on the type of adsorbent but also on the antibody titer of the sera with which adsorption was carried out. For instance, agglutinins adsorbed on charcoal, infusorial earth and alizarin showed immunological activity if the titer of the specific immune sera from which the agglutinins were adsorbed was not less than 1 : 3200. In carrying out experiments with alizarin it must be remembered that this substance is soluble in an alkaline medium, when the suspension of alizarin becomes unstable. In order to prevent the appearance of nonspecific reactions it is essential to use antigen solutions with a pH of not higher than 7.2-7.6, so that after mixing of the antigens with the suspensions of alizarin (preserved with 1% boric acid) the pH of the medium in which the agglomeration reaction is to take place shall not be greater than 6.0.

Since the immunological properties of the adsorbed antibodies were demonstrated most actively when alizarin was used as adsorbent, in the subsequent experiments only this substance was used. In the experiments we tested the immunological activity of different species of antibodies, adsorbed on suspensions of alizarin, from the following immune sera:

dysentery agglutinating serum (Flexner), titer	1 : 3200
paratyphoid B agglutinating serum, titer	1 : 3200
tularemia agglutinating serum, titer	1 : 1500
precipitating serum against serum protein of cattle, titer	1 : 10,000
precipitating serum against human serum protein, titer	1 : 10,000
influenza type A, titer of complement-fixing antibodies	1 : 640
influenza type B, titer of complement-fixing antibodies	1 : 640
"Diaferm 3" type A botulinus antitoxic serum, containing	50,000 units/ml
"Diaferm 3" type B botulinus antitoxic serum, containing	50,000 units /ml

All the sera were preserved with 1% boric acid. During investigation of the immunological activity of the antibodies we determined the minimal quantities of antigens producing an agglomeration reaction.

RESULTS

The results of the study of the immunological activity of the antibodies adsorbed on alizarin particles are shown in the table.

It will be seen from the Table that agglutinins, when adsorbed on alizarin, gave an agglomeration reaction with the homologous bacterial cells and complete antigens, but did not give an agglomeration reaction with haptens. It must be pointed out that intensive agglomeration reactions took place with complete antigens, extracted with trichloroacetic acid. The agglomeration reaction took place with complete antigens obtained by means of enzymic digestion only in the presence of high concentrations of antigen (1 : 500-1 : 1000). Precipitins against human and animal serum proteins, when adsorbed on alizarin, did not give an agglomeration reaction with homologous protein antigens (when working with human and animal blood sera as antigens, in order to prevent nonspecific reactions 1% boric acid must be added to them). The results of our experiments with precipitins against human and animal proteins were in full agreement with those obtained by Vekerdi [4]. Complement-fixing virus antibodies and botulinus antitoxins, when adsorbed on alizarin, gave agglomeration reactions with homologous antigens.

It can be seen from the table that antibodies, adsorbed on alizarin, reacted with antigens in small

Immunological Activity of Antibodies Adsorbed on Particles of Alizarin

Antigen	Type of antigen	Minimal quantity of antigen producing an agglomeration reaction	Type of antibodies adsorbed on alizarin								
			Agglutinins			Precipitins		Complement-fixing		Antitoxins	
			Parathyphoid B	Flexner dysentery	Tularemia	against human serum protein	against serum protein of cattle	against influenza virus A	against influenza virus B	type A botulinus	type B botulinus
Bacterial cells	Paratyphoid B	25 M bacterial cells/ml	+	–	–	–	–	–	–	–	–
	Flexner dysentery	The same	–	+	–	–	–	–	–	–	–
	Tularemia ***	100 M bacterial cells/ml	–	–	+	–	–	–	–	–	–
Complete* antigens	Paratyphoid B	1 : 1,600,000	+	–	–	–	–	–	–	–	–
	Flexner dysentery	1 : 800,000	–	+	–	–	–	–	–	–	–
Haptens**	Paratyphoid B	–	–	–	–	–	–	–	–	–	–
	Flexner dysentery	–	–	–	–	–	–	–	–	–	–
Protein	Human blood serum	– –	–	–	–	–	–	–	–	–	–
	Ox blood serium	– –	–	–	–	–	–	–	–	–	–
Viruses	Culture of influenza virus A ****	Not determined	–	–	–	–	–	+	–	–	–
	Culture of influenza virus B ****	Not determined	–	–	–	–	–	–	+	–	–
Toxins	Type A botulinus toxin	22,5000 lethal doses for white mice	–	–	–	–	–	–	–	+	–

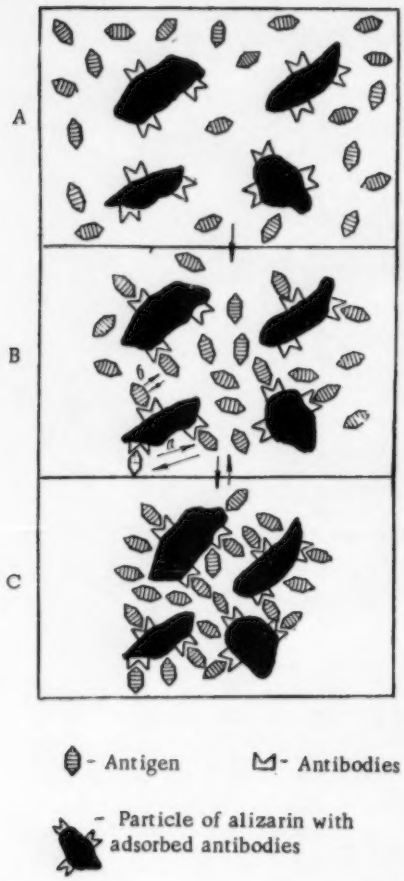
Note. The plus sign means that this antigen gives an agglomeration reaction; the minus sign means that this antigen does not give an agglomeration reaction.

* Complete antigens were extracted with trichloroacetic acid.

** Haptens were extracted by the method of A.T. Kravchenko and M. I. Sokolov (1946).

*** Vaccine strain.

**** Cultures of influenza viruses in the chick embryo.



Scheme of the agglomeration reaction of alizarin suspension antibodies. A) Appearance of the suspension antibodies in the first moment after mixing with homologous antigens; B) adsorption of antigens on alizarin suspension antibodies; C) agglomerate of alizarin suspension antibodies and homologous antigens.

concentrations (25M bacterial cells/ml, complete antigens in dilutions of 1 : 800,000 - 1 : 1,600,000), and may be used for the rapid detection of pathogenic microorganisms, complete bacterial antigens and viruses.

These experimental results thus show that the adsorption of agglutinins, antitoxins and virus complement-fixing antibodies on solid adsorbents is not accompanied by their destruction. The above-mentioned antibodies, adsorbed on solid adsorbents, retain their specific activity and are capable of giving immunological reactions with homologous antigens, which are shown in the form of agglomeration of the particles of the adsorbent. With regard to the immunological activity of precipitins against human and animal serum proteins, adsorbed on solid adsorbents, it is too early yet to form definite conclusions. The absence of an agglomeration reaction between precipitins adsorbed on solid adsorbents and homologous protein antigens gives no grounds for the assertion that the adsorption of these antibodies is accompanied by their inactivation, since immunological reactions between the above-mentioned components may not be accompanied by agglomeration of the particles of the adsorbents. It is also evident that the immunological reaction taking place between antibodies, adsorbed on solid adsorbents, and haptens is not accompanied by agglomeration of the particles of the adsorbent.

The study of the properties of suspensions of adsorbents containing adsorbed antibodies on the surface of their particles showed that, as a result of adsorption of the antibodies on the particles of the adsorbent, new compounds are formed, which combine the properties of the suspensions of adsorbents and of the serum antibodies. We therefore suggest that suspensions of adsorbents containing antibodies on their particles be called "suspension antibodies," and that the reactions between the suspension antibodies and the homologous antigens be called "suspension antibody agglomeration reactions."

The mechanisms of the suspension antibody agglomeration reaction is not yet sufficiently clear. Like other serological reactions, it does not take place in the absence of electrolytes. When the processes taking place in the agglomeration reaction between typhoid suspension agglutinins and typhoid bacilli were observed under the phase-contrast microscope, it was found that in the first moment after mixing the components the bacilli were adsorbed on the particles of the alizarin-suspended agglutinin, and the particles of suspension agglutinin, together with their adsorbed bacilli, then collected together into agglomerates. The combination of antigen with antibodies, adsorbed on alizarin, apparently takes place at the moment of adsorption of the antigen on the alizarin particles. Agglomerates of alizarin suspension antibodies are readily broken up by agitation; when the agitation ceases the alizarin suspension antibodies again form agglomerates. On the basis of our observations the mechanisms of the agglomeration reaction of alizarin suspension antibodies may be illustrated in the form of the following scheme (see Figure).

It will be seen that after mixing of the alizarin suspension antibodies, adsorption of microorganisms takes place on the surface of the alizarin particles (B). The antigens adsorbed on the particles of alizarin suspension antibodies are combined at the moment of adsorption with antibodies, are sensitized and acquire the property of agglutination with each other. The forces a, however, attracting one molecule of antigen to another on the surface

of a single alizarin particle, cannot overcome the forces of adsorption keeping these antigens on the surface of the alizarin particle. For this reason the suspension of alizarin particles, containing sensitized antigens on their surface, becomes unstable. Equilibrium in the system alizarin suspension antibodies – homologous antigens is achieved as a result of the agglutination of the antigens adsorbed on different particles of the alizarin suspension antibodies. The reaction between the sensitized antigens, adsorbed on different particles of the suspension antibodies (forces b), causes agglomeration of the particles of the suspension antibodies (c). Forces a and b are identical in nature – they are the forces which cause agglutination of antigens in the ordinary serological reactions.

There is no doubt that an important part in the agglomeration reaction of alizarin suspension antibodies is played by the electrical changes of the reacting particles and by several other factors. The description of the mechanism of the agglomeration reaction which has been given is therefore purely schematic and does not claim to provide an exhaustive explanation of this complex serological reaction.

SUMMARY

Agglutinins, virus complement-fixing antibodies and antitoxins retain their immunological activity after adsorption on solid adsorbents (alizarin etc.). Precipitins against human and animal serum protein, adsorbed on solid adsorbents do not give agglomeration reactions with homologous antigens. Antibodies, adsorbed on solid adsorbents react even with a small amount of antigen and may be used for quick detection of microorganisms and viruses. A term "suspension antibodies" is suggested for antibodies, adsorbed on solid adsorbents, and "suspension antibodies of agglomeration reactions" – for reactions between suspension antibodies and homologous antigens. The mechanism of this reaction is described.

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ISOLATION OF THE CENTER OF SPECIFIC ACTIVITY OF AN ANTIBODY FROM AN ANTISERUM HYDROLYZED WITH PAPAIN, USING AN ANTIGEN FIXED ON CELLULOSE

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(Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 76-79, November, 1960
Original article submitted October 10, 1959

It has previously been shown [4-7] that, as a result of the action of the proteolytic enzyme papain on an immune globulin, the molecule of the latter is split up into several parts, without loss of the power of this γ-globulin to react specifically with its own antigen.

All attempts to isolate from the products of proteolysis in a pure form the fragment of the globulin molecule possessing the specific antideterminant, using chemical or physicochemical methods [6, 7] for this purpose, have, however, been unsuccessful.

It accordingly appeared to be of great interest to use the specific antigen–antibody reaction to isolate the active center of the antibody from the products of proteolysis, using for this purpose an antigen fixed on an insoluble carrier (cellulose).

EXPERIMENTAL METHOD AND RESULTS

The immune sorbent in our experiments was horse albumin, fixed on cellulose by Campbell's method [3], which we modified.*

A rabbit immune serum against crystalline horse albumin was fermented with papain** for 16 hours at 37°. The papain was preliminarily activated by being allowed to stand in a buffer solution at pH 7.0, consisting of equal parts of a 0.05M solution of cystein hydrochloride, a 0.01M solution of Na-versene and a 0.3M solution of K_2HPO_4. The duration of activation was 1 hour at 42°. The quantity of papain added to the serum was 0.5 mg for each 70 mg of serum protein.

After the completion of the fermentation of the serum, it was cooled to 2°, to it was added a 0.05M solution of monoiodoacetate (papain inhibitor), and it was then subjected to rapid dialysis in the cold against physiological saline. Dialysis continued for 28-30 hours.

The experiments showed that a serum, when fermented in this way, completely lost its precipitating properties, but at the same time preserved its power of specific combination with its antigen (horse albumin), which could be judged by the ability of this serum to specifically inhibit the precipitation reaction between horse albumin and unchanged serum. In subsequent work this test has been used for detection of the specific antideterminant in a fermented serum.

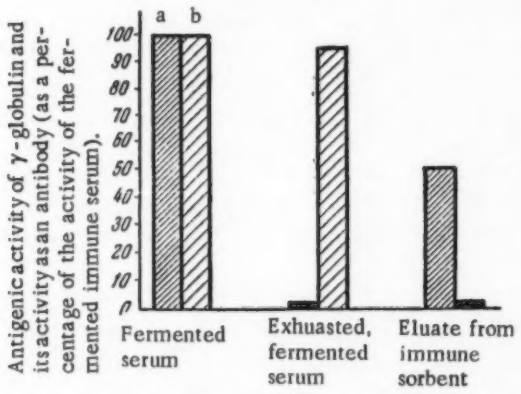
* In contrast to Campbell, the celiular tissue was not combined with p-nitrobenzylchloride but with p-nitrobenzylbromide, which has a much higher reactive power.

** The preparation of papain was obtained from the Baker Research Laboratory, London.

Protein Content of Specific Precipitate (in mg) Obtained as a Result of the Reaction between a Fermented Rabbit Immune Serum (Antigen) and an Ass's Serum Against Rabbit Globulin

Antibody	Quantity of antigen (in mg)							
	0,005	0,01	0,02	0,04	0,08	0,16	0,32	0,64
	quantity of protein in specific precipitate (in mg)							
Fermented serum	0,223	0,344	0,429	0,671	0,809	0,945	1,083	0,573
Fermented serum, exhausted with immune sorbent	0,172	0,297	0,395	0,621	0,730	0,890	0,985	0,521
Eluate from immune sorbent	—	—	—	—	—	—	—	—

Note. In the experiments 0.25 ml of antiserum, diluted 1 : 2, and a corresponding amount of antigen were used.



The presence of the antigenic determinant of the γ-globulins (b) and the antideterminant of the antibody (a) in an immune serum after fermentation with papain, in the same serum when exhausted with immune sorbent, and in the eluate from the immune sorbent.

In order to isolate the specifically active center of the antibodies, the immune serum, after fermentation as described above, was mixed with the immune sorbent for 2 hours at room temperature, after which the supernantant fluid was tested for its content of antideterminant in inhibition experiments. These experiments showed that the immune sorbent completely extracted from the serum the fragment of the γ-globulin molecule possessing specific antibody activity, as a result of which the serum, after exhaustion by the immune sorbent, lost its ability to specifically inhibit the precipitation reaction.

After the thorough removal of proteins not reacting with the immune sorbent, by means of the repeated washing with physiological saline on a Büchner funnel, elution of the specific inhibiting factor from the immune sorbent was carried out. The elution was done by acidification to pH 2.6 for 2 hours at room temperature, after which the presence of specifically active centers of the antibody was determined in the eluate.

The experiments showed that the eluate does, in fact, contain the antideterminants of the antibodies, as disclosed by its ability to specifically inhibit the precipitation reaction between the unchanged serum and its antigen.

Determination of the protein content in the eluate showed that, taking account of all the volume changes, it comprised one third of the total protein content of the antibodies in the test serum (8.6 mg antibody protein

in 1 ml of serum compared with 3.22 mg protein in 1 ml of eluate). Under these circumstances the eluate preserved about one half the inhibiting activity of the fermented serum.

In connection with these findings it was thought to be most important to discover whether the fragment of the antibody molecule which we had isolated, bearing the specific antideterminant, possessed the antigenic activity appertaining to rabbit globulin.

Experiments showed that a rabbit serum against horse albumin, subjected to fermentation and used as antigen, gave a precipitation reaction with the immune serum of an ass against rabbit globulin. After exhaustion of the fermented serum with the immune sorbent, which led to the loss of its function as an antibody, it nevertheless fully retained its ability to precipitate an ass's antiserum. Meanwhile the eluate from the immune sorbent not only did not precipitate the ass's antiserum, but also it did not inhibit the reaction of the latter with unchanged rabbit globulin (see Table).

The results obtained (see Figure) show that the specifically active center of the antibody and the antigenic determinant inherent in γ-globulin are found in different parts of the antibody molecule, which enables the specific antideterminant to be isolated in a pure form, not possessing the antigenicity of γ-globulin.

As Porter [6] reported recently, during fractionation of immune globulin, fermented with papain, on a chromatographic column, he was able to obtain three fractions, two of which contained active centers of the antibody in addition to inactive protein, and one possessed only the properties of an antigen. These results are in complete agreement with our own.

According to the findings of Treffers [8] and Wright [9], and as a result of systematic investigations carried out recently by G. N. Kryzhanovskii, L. N. Fontalin and L. A. Pevnitskii [1, 2], it has been shown that antibodies do not possess antigenic properties which differ from the antigenic properties of γ-globulin. Our findings, according to which the fragment of the antibody molecule bearing the antideterminant does not possess the inherent antigenic properties of γ-globulin, support the above investigations to a certain degree.

SUMMARY

A cellulose-fixed antigen was used to isolate the specifically active antibody center from papain-fermented antiserum. This antibody center possessed no antigenic properties characteristic of γ-globulin.

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DETECTION OF INCOMPLETE ANTIBODIES IN DOGS AFTER AFTER HOMOPLASTIC TRANSPLANTATION OF VARIOUS ORGANS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 79-85, November, 1960
Original article submitted April 25, 1959

Autoplastic transplantation of tissues such as the skin is usually a successful procedure and is widely used in plastic operations, whereas homografts as a rule die after a varying period of time after transplantation.

The reason for the different behavior of auto- and homografts is thought by many workers to be immunological differences between the tissues of the donor and recipient [1, 4, 5, 9, 12, 16 and others].

It has been shown that human erythrocytes contain about 40 different antigens, by means of which 9 immunological systems can be differentiated in man, comprising more than 300,000 individual antigenic variations. A diversity of antigenic properties has also been demonstrated for human tissues [5].

The antigenic composition of the cells and tissues of animals has received less study. There are, however, many experimental findings which give evidence of the complexity of the antigenic structure of animal tissues and of the wide variety of the antigens in animals of the same species. For instance, during the study of the antigenic structure of the dog's red cells it was found that at least four different agglutinogens were present therein [6, 11, 13, 15, 17, 18].

The existence of antigenic differences between the tissues of dogs was confirmed by experiments on repeated homotransplantation of kidneys. It was found that secondary kidney homografts died more quickly than primary, as a result of immunization of the recipient during the primary grafting [10, 16, and others].

Attempts have been made to detect specific antibodies to the donor's antigens, arising in the recipient as a result of homotransplantation. Serological investigations of dogs' sera have given contradictory results.

Yu. Yu. Voronoi [2, 3], for instance, reported the appearance of complement-fixing antibodies in the serum of dogs after grafting of a kidney.

Curtiss and Herndon [8] found agglutinins to the donor's erythrocytes in the serum of 2 of 5 dogs after homoplastic transplantation of a joint.

Simonsen [16], on the other hand, could find neither hemagglutinins nor complement-fixing antibodies in the serum of dogs after transplantation of kidneys.

Greater success was obtained in the investigations of Simonsen [16] and of Muirhead and Groves [14], when they used the agglutination reaction in a protein medium and the Coombs' test [7], for they were able to detect incomplete antibodies to the donor's erythrocytes in the serum of dogs after grafting operations.

The present experimental research was devoted to the immunological study of the sera of dogs after the homoplastic transplantation of different organs.

TABLE 1

Group of dogs	Character of homoplastic operation performed	No. of dogs examined	
		before operation	after operation
First	Grafting of a second heart with a lung or the lobe of a lung	11	5
Second	Grafting of a kidney with or without removal of the animal's own kidney	5	5
Third	Grafting of the lobe of a lung	5	2
Fourth	Creation of a crossed circulation between donor and recipient	4	4
Fifth	Complete replacement of heart and lungs, grafting of limbs or parts of the body of a puppy to an adult dog, grafting of the knee joint, and so on.	13	3
	Total number investigated	38	19

METHOD

The object used for the immunological study was the sera and erythrocytes of recipients and donors both before and at various intervals after operation.*

According to the character of the operations performed on them, the receipient dogs could be divided into five groups.

It will be clear from the details given in Table 1 that the blood of 38 pairs of donors and recipients was examined before operation. Because of the complicated nature of the operations, half the recipients died within 48 hours of operation, and so their blood was not reexamined. The serum and erythrocytes of 19 recipients whose period of survival after operation was longer than four days were tested in immunological reactions in order to detect the changes accompanying homografting.

In performing the investigations two methods were used:

1) the agglutination reaction in a saline medium [2 drops of serum (whole and diluted) + 1 drop of a 3-5% suspension of erythrocytes in physiological saline, with subsequent centrifugation for 1 minute at 2000 rpm];

2) the agglutination reaction in a protein medium [2 drops of whole or diluted serum were poured out, and then to each tube was added 1 drop of a 3-5% suspension of erythrocytes in a 20% solution of albumin. The tubes were shaken and allowed to stand for 1.5-2 hours at room temperature. To prepare the 20% albumin solution, 1 g of dry albumin from human serum was dissolved in 5 ml of 0.85% NaCl solution, after which the mixture was rendered alkaline (to pH 7.2) by the addition of a few drops of 10% NaOH solution].

The results of the hemagglutination reaction in a protein medium were read by means of a hand lens over a pearl electric light.

RESULTS

In Table 2 we give the results of the immunological investigation of the sera of 17** recipients with the donors' erythrocytes at various intervals after homografting operations.

It is clear from Table 2 that during the investigation of the recipients' sera by the agglutination reaction with the donors' erythrocytes in a saline medium, only in the dog Urs was it possible to detect agglutinins to the donor's erythrocytes on the tenth day after operation.

In the other cases no agglutinins to the donor's erythrocytes were found by this method in the recipients' serum, both before and at different intervals after the homoplastic operations.

We obtained more successful results by using the agglutination reaction with the donor's erythrocytes in a

* The operations were performed by V. P. Demikhov in the laboratory for transplantation of organs.

** The results of investigation of the sera of two recipients, dying on the fifth day after transplantation of the head of the femur, are not given in the table. No changes were found in the sera of these dogs.

TABLE 2

Results of the Reaction of Agglutination of the Donors' Erythrocytes by the Recipients' Sera after Homoplastic Transplantation Operations

Recipient	Character of homo-transplantation operation	Length of survival after operation (in days)	Time of investigation of serum	Method of investigation: agglutination reaction in saline solution — Dilutions of sera: whole	1:2	1:4	1:8	1.16	1:32	agglutination reaction in protein solution — Dilutions of sera: whole	1:2	1:4	1:8	1:16	1:32
Starik	Grafting of a second heart and a lung	10	Before operation	—	—	—	—	—	—	+	++	++	±	±	—
			On 7th day after operat.	—	—	—	—	—	—	+++	++	++	++	+(+)	+
			On 9th day after operat.	—	—	—	—	—	—	+++	+++	+(+)	+(+)	+	±
Stroinyi	Grafting of a second heart and of a lobe of a lung	14	Before operation	—	—	—	—	—	—	+	—	—	—	—	—
			On 7th day after operat.	—	—	—	—	—	—	++	++	++	±	—	—
			On 10th day after operat.	—	—	—	—	—	—	+✦	+++	+++	++	+	±
Urs	Grafting of second heart and a lung	13	Before operation	+	±	—	—	—	—	+++	+++	+++	++	+	±
			On 10th day after operat.	+	+	+	+	—	—	+++	+++	+++	++	+	±
Zheltyi	The same	8	Before operation	—	—	—	—	—	—	++	+	±	—	—	—
			On 4th day after operat.	—	—	—	—	—	—	+	+	—	—	—	—
			On 6th day after operat.	—	—	—	—	—	—	++	+	±	—	—	—
Khromoi	» »	5	Before operation	—	—	—	—	—	—	+(+)	±	—	—	—	—
			On 5th day after operat.	—	—	—	—	—	—	+	—	—	—	—	—
Buket	Grafting of a kidney after removal of the recipient's own kidneys	11	Before operation	—	—	—	—	—	—	+	+	±	—	—	—
			On 5th day after operat.	—	—	—	—	—	—	+	+	±	—	—	—
			On 11th day after operat.	—	—	—	—	—	—	++	+++	++	++	+	—
Chernysh	Grafting of a kidney	45	Before operation	—	—	—	—	—	—	—	—	—	—	—	—
			On 8th day after operat.	—	—	—	—	—	—	—	—	—	—	—	—
			On 19th day after operat.	—	—	—	—	—	—	+	±	—	—	—	—
			On 32nd day after operat.	—	—	—	—	—	—	+	+	—	—	—	—
			On 45th day after operat.	—	—	—	—	—	—	+(+)	+	+	—	—	—

TABLE 2 (continued)

Recipient	Character of homotransplantation operation	Length of survival after operation (days)	Time of investigation of serum	Method of investigation: agglutination reaction in saline solution — Dilutions of sera: whole	1:2	1:4	1:8	1:16	1:32	agglutination reaction in protein solution — Dilutions of sera: whole	1:2	1:4	1:8	1:16	1:32
Polkan	Grafting of a kidney	9	On 6th day after operat.	–	–	–	–	–	–	++	++	–	–	–	–
Chernyi	The same	14	Before operation	–	–	–	–	–	–	±	±	–	–	–	–
			On 8th day after operat.	–	–	–	–	–	–	–	–	–	–	–	–
			On 12th day after operat.	–	–	–	–	–	–	±	±	±	±	–	–
Tuzik	The same	9	Before operation	–	–	–	–	–	–	±	–	–	–	–	–
			On 5th day after operat.	–	–	–	–	–	–	±	–	–	–	–	–
Lokhmach	Grafting of the lower lobe of a lung	45	Before operation	–	–	–	–	–	–	+	±	–	–	–	–
			On 4th day after operat.	–	–	–	–	–	–	+	±	–	–	–	–
			On 11th day after operat.	–	–	–	–	–	–	±	±	–	–	–	–
Recipient No. 2	The same	11	Before operation	–	–	–	–	–	–	±	–	–	–	–	–
			On 6th day after operat.	–	–	–	–	–	–	–	–	–	–	–	–
Kerzon	Grafting of a knee joint	more 12	Before operation	+	±	–	–	–	–	+	+	–	–	–	–
			On 11th day after operat.	+	±	–	–	–	–	+	+	±	–	–	–
Dog No. 1	Creation of a crossed circulation	9	Before operation	–	–	–	–	–	–	+	±	±	–	–	–
			On 5th day after operat.	–	–	–	–	–	–	+	–	–	–	–	–
			On 7th day after operat.	–	–	–	–	–	–	±	–	–	–	–	–
Dog No. 2		9	Before operation	–	–	–	–	–	–	+	±	–	–	–	–
			On 5th day after operat.	–	–	–	–	–	–	–	–	–	–	–	–
			On 7th day after operat.	–	–	–	–	–	–	+	–	–	–	–	–

TABLE 2 (continued)

Recipient	Character of homotransplantation operation	Length of survival after operation (days)	Time of investigation of serum	Method of investigation										
				agglutination reaction in saline solution					agglutination reaction in protein solution					
				Dilutions of sera										
				whole	1:2	1:4	1:8	1:16	1:32	whole	1:2	1:4	1:8	1:16	1:32
Ryzhii	Creation of a crossed circulation	4	Before operation	—	—	—	—	—	—	—	—	—	—	—	—
			On 4th day after operat.	—	—	—	—	—	—	—	—	—	—	—	—
Malysh		4	Before operation	—	—	—	—	—	—	—	—	—	—	—	—
			On 4th day after operat.	—	—	—	—	—	—	—	—	—	—	—	—

Conventional signs: +++ precipitate of erythrocytes fills the whole of the bottom of the tube in the form of an umbrella with winding edges, sometimes marked with radial stripes; ++ the precipitate fills almost the entire bottom of the tube and clumps of agglutinated erythrocytes are seen; + part of the erythrocytes appear as a homogeneous precipitate, the rest in the form of clumps or agglutinates. The edge of the precipitate is uneven. The surface is rough. (+) The greater part of the erythrocytes appear as a homogeneous precipitate and there are a few clumps. ± Doubtful agglutination. The edges of the precipitate are not quite smooth. – Erythrocytes are evenly deposited at the bottom of the tube; a homogeneous precipitate with a clear, smooth border.

20% albumin solution. By using this method we were able to demonstrate the presence of agglutinins to the donor's erythrocytes in several recipients and to observe an increase in their titer after homografting operations.

A second heart was grafted on to the dog Starik, with a lung in the thorax. The dog survived for ten days after operation.

It can be seen from Table 2 that before operation agglutinins to the donor's erythrocytes were found in the recipient's serum to dilutions of 1 : 4. On the seventh day after operation the titer of agglutinins to the donor's erythrocytes was increased. There was a well-marked reaction of agglutination of the donor's erythrocytes to a dilution of the serum of 1 : 32. The same results were obtained on the ninth day. The recipient's serum did not agglutinate its own erythrocytes.

The serum of the dog Stroinyi before operation agglutinated the donor's erythrocytes in albumin solution only in the undiluted form. On the seventh day after grafting of the second heart with a lobe of the lung into the thorax, the agglutinin titer rose to a dilution of 1 : 4, and on the tenth day after operation a clear reaction of agglutination of the donor's erythrocytes took place to a dilution of 1 : 16.

In the serum of the dog Urs, into whose thorax a second heart, with a lung, was grafted, in a saline medium agglutinins to the donor's erythrocytes were found in a titer of 1 : 8 on the tenth day after operation.

When investigated in a protein medium, the agglutination reaction was more clearly apparent and the agglutinin titer was higher (to a dilution of 1 : 16). No differences were found, however, in the agglutinin titer before and on the tenth day after operation.

A donor's kidney was grafted on to the dog Buket after its own kidneys had been removed. The recipient died from uremia on the 11th day after operation. It is clear from Table 2 that the recipient's serum before operation and on the fifth day after operation feebly agglutinated the donor's erythrocytes in the undiluted form and in a dilution of 1 : 2. On the 11th day after operation agglutinins to the donor's erythrocytes were found in the recipient's serum to a dilution of 1 : 16, whereas the recipient's own erythrocytes and the erythrocytes of other dogs were agglutinated by the serum only to a dilution of 1 : 4.

Grafting of a decapsulated kidney was performed on the dog Chernysh, without removal of the animal's own kidneys. The dog remained in a satisfactory condition. On the 45th day after operation an exploratory laparotomy was performed. The grafted kidney was found to be enlarged four-or fivefold and the renal artery was thrombosed. It is evident from Table 2 that the recipient's serum before operation and on the eighth day after operation did not agglutinate the donor's erythrocytes. Only on the 19th day after operation were agglutinins to the donor's erythrocytes found in whole serum, their titer rising very slowly to 1 : 4 on the 45th day.

Agglutinins to the donor's erythrocytes were found to a dilution of 1 : 2 in the serum of the dog Polkan on the sixth day after homoplastic transplantation of a kidney. Unfortunately this serum was not tested before operation.

It is clear from Table 2 that the appearance of agglutinins was not observed in all the animals.

The titer of agglutinins to the donor's erythrocytes was not increased in the recipients Zheltkyi and Khromoi, whose sera were tested on the 4th-6th day after operation.

In the serum of the recipient Chernyi, tested on the eighth and 12th day after operation, and that of the recipient Tuzik, tested on the fifth day after operation, agglutinins to the donor's erythrocytes were absent.

The dog Lokhmach, on which the operation of grafting of a lower lobe of the donor's lung was performed, survived for 45 days after operation. When it was examined post mortem, the grafted lobe was found to be almost completely absorbed. Since the donor was not kept alive, no immunological investigations were carried out after the 11th day. Tests of the serum on the fourth and 11th day after operation showed the absence of agglutinins to the donor's erythrocytes.

No increase in the agglutinin titer was observed in the dog Kerzon on the 11th day after grafting of a knee joint, or in recipient No. 2 after grafting of a lung. No significant changes were found, moreover, in the sera of two pairs of dogs after the creation of a crossed circulation.

Immune agglutinins were thus found in the serum of five recipient dogs on which a heart and lung, or a kidney had been grafted, out of the 19 dogs investigated after various homografting operations, in a titer of between 1 : 4 and 1 : 32, to the erythrocytes of the corresponding donors, the appearance of which may be accounted for by immunization of the recipient with antigens of the donor's tissues.

These agglutinins evidently belong to the group of incomplete antibodies, for they are best detected in a protein medium by the conglutination method.

The absence of immunological changes in the serum of the majority of recipients after homografting operations may evidently be explained by the fact that the sera were tested on the fourth, the fifth and, only in a few cases, on the sixth day after operation, whereas the immunological reaction of the body to the introduction of foreign antigens is known to develop only seven or more days after the introduction of these antigens.

In some cases, despite the comparatively long survival period of the recipient after grafting of the organs, no antibodies to the donor's erythrocytes could be found in its serum. These cases may be explained by the peculiarities of the immune reaction of the recipient, by the specific features of the antigenic structure of the grafted organs, and also, possibly, by the close resemblance between the antigenic properties of the tissues of donor and recipient.

SUMMARY

Comparative immunological examination of sera obtained from recipient dogs before transplantation and at various periods after the homotransplantation of various organs demonstrated that immune agglutinins to the donor's erythrocytes may appear in the recipients' sera. These agglutinins belong to the group of incomplete antibodies, since they are best detected by the conglutination method. The appearance of hemagglutinins in the serum of recipients points to antigenic differences between the donor's and recipient's tissues has the basis of tissue incompatibility.

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PRODUCTION OF AN ANTIRONIDASE HORSE SERUM AND ITS ACTION ON METASTASIZATION OF A BROWN-PEARCE CARDINOMA IN RABBITS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50
No. 11, pp 86-90, November, 1960
Original article submitted May 20, 1960

In previous work we showed depression of metastasization of a Brown-Pearce carcinoma in rabbits, after removal of the primary tumors, under the influence of an antironidase goat serum. Since the subject of the investigation is of practical as well as theoretical interest, an extension of the work in this direction is extremely important.

The object of the present investigation was to ascertain whether an antironidase serum, obtained by immunization of horses, possesses an inhibiting action on metastasization of a Brown-Pearce carcinoma.

METHOD

As donors of antironidase sera we used two horses (mare No. 11 and stallion No. 13). Immunization* of the animals was done in two cycles. The first cycle consisted of seven intramuscular injections of ronidase in doses of 1.5 to 8 g per injection. As a result of the first cycle of immunization, however, we were unable to induce a sufficiently high level of antibody production. The highest titer of antironidase antibodies in the McClean–Smirnova reaction was 1 : 64.

The subsequent immunization of the horses was temporarily postponed on account of the development of areas of infiltration at the sites of injection of the enzyme into the animals.

Six months later the horses were reimmunized by means of four intramuscular injections of 6, 15, 20 and 22.5 g ronidase, with intervals of 5-8 days between injections. During both immunization and reimmunization observations were made on the trend of antibody formation. At the moment of appearance of antibodies in a sufficiently high titer (on the ninth day after the last injection or ronidase) bleeding was carried out and the sera prepared.

The sera obtained were used for experiments after 2.5 months. The serum of horse No. 13, tested at this period by the McClean reaction, suppressed the activity of the homologous enzyme ronidase in a dilution of 1: 4096, testicular enzyme of the rabbit and guinea pig in a dilution of 1 : 256, and testicular enzyme of the rat in a dilution of 1 : 64. The serum of horse No. 11.depressed the activity of these enzymes in the following dilutions respectively: ronidase 1 : 1024, testicular enzyme of the rabbit and rat 1 : 128 and guinea pig enzyme 1 : 256.

* Immunization was carried out at the Gamaleya Institute under the supervision of A. V. Ushakova, to whom we express our gratitude.

TABLE 1

Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Tumor and Injection of Horse Sera (Sacrificed on the 21st Day after Inoculation of the Tumor)

Group of rabbits	Tumor	Serum	No. of rabbits	Mean number of affected organs per rabbit	Mean number of metastase* per affected organ	Weight of omentum (in g)	Weight of spermatic cord (in g)
First	Removed	Antironidase	4	3.2	8.3	15.	6.0
Second	"	Normal	4	5.7	20.1	25.7	6.0
Third	"	No serum injected	4	3.0	25.1	37	10.1
Fourth	Not removed	The same	4	4.7	44.6	24.2	

* In counting the number of metastases per affected organ, the metastases in the spermatic cord and omentum were disregarded.

Both sera were used in the experiments, which were conducted as follows: 39 male chinchilla rabbits, weighing 2.5-3.2 kg, were given an injection of 1 ml of a 25% suspension of a Brown-Pearce tumor into the left testicle. All the animals were then subsequently divided into three groups of equal weight. On the sixth day of development of the tumor, the rabbits of the first group received an intravenous injection of 6 ml of antironidase horse serum. The rabbits of the second group received an injection of the same volume of normal horse serum. The animals of the third group received no serum whatever.

On the seventh day the left testicle of 35 rabbits was removed and examined for the presence of a tumor. In four rabbits of the third group (Nos. 5, 90, 65 and 36) the testicle was not removed, and these animals then formed the fourth group.

On the next day, the eighth after transplantation of the tumor, 5 ml of the corresponding serum was injected intratesticularly into the rabbits of the first and second groups. Subsequently, at intervals of 2 days, each rabbit received a further four injections, each of 6 ml, alternately into the right and left posterior muscles of the thigh. Thus, altogether 6 injections were given, 2 intravenously and 4 intramuscularly.

On the 21st day after inoculation of the tumor, 16 rabbits, i.e., 4 from each group, were sacrificed. The remaining rabbits were sacrificed on the 36th day.

The effectiveness of the action of the sera on metastasization was estimated by the degree to which the organs were affected by metastases. As an index of the latter we used the number of organs affected by metastases, the average number of metastases in one affected organ and the weight of the omentum and the spermatic cord. The technique of counting the metastases in the organs was described in detail in the previous paper [1].

The results obtained at sacrifice of the animals on the 36th day were treated statistically by the Fisher-Student method. The differences were considered significant if the value of P was not greater than 0.05.

RESULTS

As the figures in Table 1 show, on the 21st day after transplantation of the tumor, in the rabbits from which the primary focus had been removed and which had received injections of normal horse serum (second group) and also in the rabbits which had received no treatment whatever (fourth group) the average number of affected organs was 5.7 and 4.7 respectively.

In the rabbits receiving antironidase serum (first group) the value of this index at this time was lower (3.2), although it did not differ from that in the group of animals not receiving serum after removal of the tumor (third group), for which it was (3.0).

Comparison of the mean indices of the number of metastases per affected organ shows that the density of metastasis formation in the organs of the experimental group (first group) was the lowest (8.3) in relation to all

TABLE 2

Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Tumor and Injection of Horse Sera (Sacrificed on the 36th Day after Inoculation of the Tumor)

Group of rabbits	Tumor	Serum	Rabbit No.	Number of affected organs	Mean No.* of metastases per affected organ	Omentum		Spermatic cord	
						degree of involvement	wt. (in g)	degree of involvement	wt. (in g)
First	Removed	Antironidase	9	—	—	—	24,0	—	1,5
			12	1	—	++	8,0	—	1,0
			8	2	2,0	—	11,0	+	3,0
			54	5	4,3	+	34,5	+	11,0
			42	7	4,6	+	19,0	+	5,0
			30	8	25,6	+	12,5	+	55,0
			7	9	11,4	++	40,0	+	7,0
			2	9	15,0	++	74,5	++	26,5
		Mean		5,1	7,9		27,9		13,7
Second	Removed	Normal	16	1	1,0	—	13,0	—	2,0
			24	1	1,0	—	8,0	—	2,0
			23	4	12,5	+	15,0	+	60,0
			6	6	26,6	+	41,0	++	45,5
			52	7	30,2	++	74,0	++	67,0
			81	8	34,8	+++	141,0	++	35,0
			70	10	47,4	+++	225,0	++	76,0
			75	11	84,5	+++	190,0	++	28,0
			57	11	162,3	+++	162,0	++	55,0
		Mean		6,5	44,5		96,55		41,2
Third	Removed	No serum injected	61	7	20,2	+	12,0	+	5,5
			1	8	46,8	++	65,0	++	47,0
			83	8	94,4	++	50,0	++	60,0
			55	11	48,5	+++	150,0	++	60,0
			87	9	153,0	+++	185,0	++	36,0
		Mean		8,6	72,6		92,4		41,7

Conventional signs: — metastases absent; + few metastases (from 1 to 10); ++ multiple metastases (from 11 to 50); +++ metastases could not be counted (large, confluent nodules of metastases).

* In counting the mean number of metastases per affected organ, the metastases in the spermatic cord and omentum were disregarded.

the control group (including the third group), in which the average number of metastases was 20.1 (in the second group), 25.1 (in the third group) and 44.6 (in the fourth group).

Involvement of the omentum was also least pronounced in the animals of the first (experimental) group (15 g against 25.7, 37.0 and 24.2 g in the controls).

The development of the tumor in the spermatic cord was more marked in the third group (average weight 10.1 g) than in the first and second groups (6.0 g).

Since in the fourth group (technical control) the testicle with the tumor was not removed, no comparison of the degree of involvement of the spermatic cord was made. It should be pointed out, however, that in all the rabbits of this group the tumor developed and reached considerable dimensions (weight of the testicle with the tumor in rabbits No. 5 – 24 g, No. 36 – 18 g, Nos. 90 and 65 – 12 g). This indicates that the strain of Brown-Pearce tumor which we used possessed high virulence.

In Table 2 we give the results showing the degree of metastasization of the Brown-Pearce carcinoma in the rabbits at the time of their sacrifice on the 36th day. It should be pointed out that three rabbits died earlier than the stated period (rabbit No. 57 on the 24th day, No. 87, on the 25th day and No. 70 on the 35th day). Since these rabbits died as a result of intensive metastasization of the tumor, we considered that they could be included in this table. One rabbit (No. 56) died from an accidental cause and was not included in the experiment.

The experimental results also showed that the highest number of affected organs (8.6 of the 12 organs examined) was observed in the third group of animals, which received no serum, and the lowest was found in the first group – in the rabbits receiving antironidase serum (5.1 organs). In the rabbits of the second group, which received injections of normal horse serum, the average number of affected organs was 6.5.

The smallest number of metastases per affected organ was observed in the rabbits of the experimental group – 7.9, whereas in the second group, after injection of normal horse serum, the number of metastases was 47.5 and in the third group, in which the rabbits received no serum, it was 72.6. The statistical differences between the results for the first and second, and the first and third groups are significant (in the first case P = = 0.053, in the second P = 0.005). Meanwhile, the injection of normal serum led to no significant decrease in the number of metastases. The difference between the mean values for the second and third groups is not significant (P = 0.384). This demonstrates that the degree of involvement of the organs with metastases was least after injection of antironidase serum.

The same relationship was observed when a comparison was made of the weight of the omentum and of the spermatic cord in the experimental and control animals (since the weight of the omentum and of the spermatic cord depends on the degree of development of metastases of the tumor therein). The weight of the omentum in the first group (experimental) was 27.9 g, in the second group 96.5 g and in the third group 92.4 g. The probability of a chance difference between the indices of the first and second and of the first and third groups is significant (in the first case P = 0.036 and in the second P = 0.030), but that between the indices of the second and third groups is not significant (P = 1). The weight of the testicle in the rabbits of the first group was 13.7 g, in the second group 41.2 g and in the third group 41.7 g. The difference between the indices in the first and second groups is significant (P = 0.024), whereas that between the indices of the second and third control groups is not significant (P = 1.0).

The injection of antironidase horse serum thus affords considerable protection against the development of metastases. This was reflected in the decrease in the number of metastases in the organs of the animals receiving antironidase serum by roughly 5.6 times compared with animals receiving injections of normal horse serum and by 9.2 times compared with the animals receiving no treatment. Moreover, in the animals receiving antironidase serum, metastasis formation in the omentum and spermatic cord was minimal. A slight decrease in the incidence of involvement of the organs by metastases was also observed.

SUMMARY

Antironidase serum was obtained by immunizing 2 horses with ronidase. This serum was tested on rabbits after removal of a Brown-Pearce tumor. Metastasization was depressed by this serum: there was a reduction of the mean number of metastases in the affected organs, of the degree of metastasization in the omentum and spermatic cord, as well as of the number of organs affected by metastases.

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CHANGES IN THE DISTRIBUTION OF HYALURONIDASE IN ANIMALS AFFECTED BY TUMORS AS A RESULT OF THE ACTION OF AN ANTITUMOR SERUM

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 90-95, November, 1960
Original article submitted July 27, 1959

In papers by several authors [2-5] there are reports that the development of a neoplasm in animals and man is accompanied by the appearance of the enzyme hyaluronidase in the blood and tissues. It has also been observed that hyaluronidase is a factor responsible for the infiltrating character of the growth of a neoplasm and for the degree of its metastasization.

The aim of our research was to investigate the appearance of hyaluronidase and its distribution in the organs during the development of a tumor, and to study the changes in this distribution under the influence of certain therapeutic factors.

METHOD

Rabbits were used as experimental animals. Preliminary experiments on healthy animals showed the absence of hyaluronidase in their organs and tissues. Further experiments were carried out on 59 rabbits, of which 44 were experimental and 15 control.

A 20% supspension of cells of a Brown-Pearce tumor in physiological saline was inoculated into the left sex gland of all the rabbits. On the 12th day, when growth of the tumor could be observed in the primary focus, together with involvement of the internal organs, injections of Prof. V. A. Chepurin's [5] anticancer serum were begun, in a dose of 0.3 ml/kg body weight. The serum was injected subcutaneously twice a week. At different periods of treatment animals were sacrificed and their organs and tissues investigated for their hyaluronidase content by the McCline–Smirnova method. Extracts of the various organs were mixed in definite proportions with hyaluronic acid and incubated for 30 minutes. After cooling, 15% acetic acid was added, and the reaction was regarded as positive (indicating the presence of hyaluronidase in the organs) if no mucin clot was formed in the precipitate. If a clot appeared, the reaction was considered to be negative. In order to denote involvement of the organs with metastases and the presence therein of hyaluronidase, certain conventional signs were adopted (see Table 1).

At the same time blood films were periodically taken from the animals for the differential white cell count, and blood was also taken for determination of the total white cell count.

RESULTS

It was shown that the largest quantity of hyaluronidase was present in the organs and tissues of the animals of the control group, which received no anticancer serum. These animals also showed a total leukocytosis, and the leukocyte formula revealed a neutrophilic leukocytosis and lymphocytopenia. The animals of the control

TABLE 1

Degree of Metastasization in Organs and Their Content of Hyaluronidase in Rabbits Not Receiving Anticancer Serum (Controls)

Rabbit No.	wt. (in g) before inoculation	wt. (in g) at the moment of death	Time of death (sacrifice) of animal	Material investigated: heart	lung	dia-phragm	omentum	gastric mucosa	spleen	liver	mesen-tery	adrenal	kidney	perito-neum	bladder	sex gland	blood serum
1	2200	1800	13th day (sacrificed)	++	++	++	+++	0	0	++++	+++	0	++++	++++	++	++++	0
				2	2	2	3	0	0	5	4	0	5	5	2	4	5
2	2220	1850	13th day (sacrificed)	++	++	++	+++	0	0	++++	+++	0	++++	++++	++	++++	0
				2	2	2	3	0	0	5	4	0	5	5	2	4	5
3	2150	1900	14th day (sacrificed)	++	+++	+++	+++	0	0	++++	++	0	++++	++	++	+++	0
				2	3	3	3	0	0	5	3	0	4	2	2	3	5
4	2020	1500	14th day (sacrificed)	0	++	0	0	0	0	++++	++	0	+++	+++	0	++	0
				0	2	0	0	0	0	5	3	0	4	3	0	2	5
5	1900	1555	15th day (died)	0	++	0	0	0	0	+++	+++	0	+++	0	0	+++	0
				0	2	0	0	0	0	5	3	0	4	0	0	3	5
6	1850	1600	15th day (died)	0	0	0	++	0	0	++++	++	0	+++	++	0	+++	0
				0	0	0	3	0	0	5	3	0	4	2	0	3	5
7	2150	1950	16th day (died)	0	++	++	+++	0	0	++++	0	0	+++	++	0	++++	0
				0	2	0	4	0	0	5	0	0	4	2	0	5	0
8	1950	1750	16th day (died)	++	++	++	+++	0	0	++++	+++	0	++	0	0	+++	0
				2	2	2	4	0	0	5	3	0	3	0	0	4	5
9	1800	1600	17th day (sacrificed)	++	++	++	+++	0	0	++++	++	0	+++	++	++	+++	0
				2	2	2	4	0	0	5	2	0	4	2	2	4	5
10	1900	1750	17th day (sacrificed)	++	0	++	+++	0	0	++++	++	0	+++	+++	0	++++	0
				2	0	2	4	0	0	5	2	0	4	2	0	5	5

TABLE 1 (continued)

Rabbit No.	wt. (in g) before inoculation	wt. (in g) at the moment of death	Time of death (sacrifice) of animal	Material investigated: heart	lung	dia-phragm	omentum	gastric mucosa	spleen	liver	mesen-tery	adrenal	kidney	peri-toneum	bladder	sex gland	blood serum
11	2000	1800	18th day (died)	++	++	++	+++	0	0	++++	++	0	++	+++	0	+++	0
				2	2	4	4	0	0	5	2	0	2	3	0	4	5
12	2100	1850	20th day (died)	++	0	+++	+++	0	0	++++	+++	0	+++	+++	0	++++	0
				2	0	3	4	0	0	5	3	0	4	3	0	5	5
13	2050	1600	25th day (died)	0	++	++++	++++	0	0	++++	++	0	+++	++	0	0	0
				2	0	5	5	0	0	5	2	0	4	2	0	0	5
14	2120	1550	29th day (died)	0	++	+++	++++	0	0	++++	++	0	+++	++	0	0	0
				0	2	4	5	0	0	5	2	0	3	2	0	0	5
15	1960	1500	30th day (sacrificed)	+++	++	+++	++++	0	0	++++	0	0	++	++	0	+++	0
				2	2	3	5	0	0	5	0	0	3	2	0	4	5

Legend: + few metastatic nodes present; ++ groups of nodes present; +++ considerable number of metastases; ++++ whole organ affected. Hyaluronidase content of organs: 0) absent; 1) weakly positive reaction; 2) positive in the first two tubes; 3) in the first three; 4) in the first four; 5) strongly positive reaction, hyaluronidase present in all five tubes.

TABLE 2

Degree of Metastasization of Organs and Their Content of Hyaluronidase in Rabbits Receiving Anticancer Serum (Experimental Animals)

Rabbit No.	wt.,(in g) before inoculation	wt.,(in g) at the moment of death	Time between inoculation tumor + start of treat.,(days)	No. of injections of serum	Time of sacrifice of animal (days after begin. of treat.)	Material investigated													
						heart	lung	diaphragm	omentum	gastric serosa	spleen	liver	mesentery	adrenal	kidney	peritoneum	bladder	sex gland	blood serum
1	2 200	1 650	12	1	15th	0	++	++	++	0	0	++++	++	0	+++	++		++++	0
						0	2	3	3	0	0	5	2	0	4	2		4	5
2	2 150	1 750	12	1	17th	0	++	++	+++	0	0	++++	++	0	+++	++		++++	0
						0	2	2	3	0	0	5	2	0	4	2		4	5
3	2 200	1 800	12	1	18th	0	++	+++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	3	0		2	5
4	1 980	1 550	12	2	19th	0	++	++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	2	0		2	4
5	1 950	1 800	12	2	19th	0	++	++	0	0	0	+++	++	0	++	0		++	0
						0	2	2	0	0	0	4	2	0	2	0		2	4
6	2 000	1 850	12	2	20th	0	0	++	0	0	0	+++	++	0	++	0		++	0
						0	0	2	0	0	0	3	2	0	2	0		2	4
7	2 100	1 900	12	2	20th	0	0	0	0	0	0	+++	++	0	++	0		++	0
						0	0	0	0	0	0	3	2	0	2	0		2	4
8	2 180	2 100	12	3	21st	0	0	0	0	0	0	++	0	0	++	0		+++	0
						0	0	0	0	0	0	2	0	0	2	0		4	4
9	2 140	2 000	12	3	22nd	0	0	0	0	0	0	0	0	0	+++	++		+++	0
						0	0	0	0	0	0	0	0	0	4	3		4	4
10	2 020	2 000	12	4	23rd	0	0	0	0	0	0	++	0	0	++	++		+++	0
						0	0	0	0	0	0	2	0	0	3	3		5	4

TABLE 2 (continued)

Rabbit No.	wt (in g) before inoculation	wt (in g) at the moment of death	Time betw. inocul. tumor and start of treat. (days)	No. of injections of serum	Time of sacrifice of animal (days after begin. of treat.)	Material investigated: heart	lung	diaphragm	omentum	gastric serosa	spleen	liver	mesentery	adrenal	kidney	peritoneum	bladder	sex gland	blood serum
11	2 000	2 000	12	4	23rd	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 0	++ / 3		+++ / 3	0 / 4
12	2 150	2 140	12	4	24th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	++ / 2	+ / 0		+++ / 3	0 / 4
13	2 100	2 120	12	5	25th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	0 / 0		++ / 2	0 / 3
14	2 200	2 180	12	6	29th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2		++ / 3	0 / 2
15	2 300	2 250	12		30th	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	0 / 0	++ / 2	0 / 0	0 / 0	0 / 0	0 / 2		+++ / 3	0 / 2

group lost 30-40% of their original weight (Table 1). In the animals of the experimental group the largest quantity of hyaluronidase was observed in those cases in which the animals received only one or two injections of anticancer serum. As the number of injections of anticancer serum increased in these animals, the metastases disappeared from their organs and their hyaluronidase content became smaller until it reached a minimum value, and in animals which received 5-6 injections of the therapeutic serum the hyaluronidase disappeared altogether (Table 2). The duration of survival of these animals increased and they ceased to lose weight. Study of their blood picture showed a decrease in the original leukocytosis, which gave way in the course of treatment to a reduction in the total white cell count but in the leukocyte formula the neutrophilic leukocytosis was gradually replaced by a lymphocytosis.

SUMMARY

An attempt was made in this work to trace the dynamics of hyaluronidase distribution in the body during the development of a Brown-Pearce tumor, and the changes in the hyaluronidase level in the organs following administration of anticancer serum. Preliminary experiments on healthy animals demonstrated the absence of hyaluronidase in their blood and organ tissues. With pronounced metastases, a marked positive reaction to the presence of hyaluronidase was seen in the blood serum and extracts of various organs (liver, kidneys, gonads, etc.) of experimental animals. With gradual resorption of the tumor nodes, resulting from the action of V. A. Chepurin's cancer antiserum, the amount of hyaluronidase in the organs and tissues declined; after the resolution of the nodes, hyaluronidase disappeared completely. As time went on some changes were also noted in the other indices in connection with the cancer antiserum administration. The blood picture showed a tendency to normalization, and the loss of weight, which was initially marked, was arrested.

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THE EFFECT OF PNEUMOTHORAX ON METASTASIZATION OF A BROWN-PEARCE TUMOR IN THE LUNGS OF THE RABBIT

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp 95-99, November, 1960
Original article submitted November 16, 1959

Experimental researches conducted in A. D. Speranskii's laboratories have shown that the course of metastasization is dependent upon the place and time of application of a superimposed nerve injury and also on the character of such an injury [6, 7, 8, 13, 16-19]. By changing the trophic state of the tissue in the corresponding organs, these factors create conditions favoring the development or nondevelopment of metastases.

Our work was undertaken in accordance with the planned further development of these researches. We studied the role of changes in the functional state of the lungs in the process of metastasis-formation, on the assumption that a change in the functional state leads to corresponding trophic changes and, hence, to changes in the growth of the tumor.

In the accessible literature, both Soviet and non-Soviet, we found no work on the study of metastasization in the lungs under different functional conditions.

METHODS

The present investigation was conducted on 102 male rabbits weighing 2.0-2.5 kg, inoculated with a Brown-Pierce carcinoma. In experimental oncology it is known that the highest proportion of successful inoculations and of metastasization in the internal organs is given by intratesticular transplantation of a Brown-Pearce carcinoma [2, 11, 15, 21, 23]. For this reason, for the study of the metastasization of this tumor in the lungs we used intratesticular transplantation. The changes in the functional state of the lungs were brought about by the induction of a unilateral artificial pneumothorax.

We conducted two series of experiments. In the first series 82 rabbits were used, 42 experimental and 40 control animals. The experimental rabbits underwent induction of an artificial tension pneumothorax on the right side. For this purpose 75 cc of air was introduced into the pleural cavity by means of a Kachkachev apparatus. One hour after induction of the penumothorax, a Brown-Pearce tumor was transplanted into the left testicle of all the rabbits (experimental and control) in a dose of 0.5 ml of a 20% tumor emulsion. Throughout the subsequent duration of the experiment, four insufflations of air were given into the right pleural cavity of each experimental rabbit, in volumes of 75 cc at intervals of 5 days, in order to maintain the lung in a collapsed condition. On the 26th day of the experiment all the rabbits were sacrificed and autopsied in order to study the process of metastasis formation. The development of metastases was determined in accordance with the presence of tumor nodules visible to the naked eye. Metastasization was studied in the mesentery and the serous membrane of the large and small intestine, the parietal peritoneum, the diaphragm omentum, kidneys, adrenals, liver, and lungs. For convenience of comparison of the degree of metastasization in the various organs of the

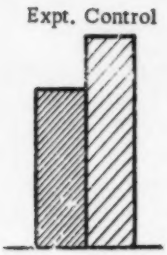

Fig. 1. Degree of involvement of organs by metastases of a Brown-Pearce tumor. Closely shaded part – degree of metastasization in the pneumothorax experiment; lightly shaded part – degree of metastasization in controls.

Fig. 2. Distribution of metastases of a Brown-Pearce tumor in the lungs after induction of an artificial tension pneumothorax on the right side.

experimental and control animals, we used a conventional numerical notation for the metastases in accordance with their size and number. As regards size: small – under 0.2 cm, average – from 0.2 to 0.5 cm and large – over 0.5 cm; as regards number: few – under ten, average – from 10 to 30, and multiple – over 30. The conventional numerical notation was as follows: few small metastases were denoted by 1 point, an average number of small metastases – 2, multiple small metastases – 3 points; few average-sized metastases – 2 points, an average number of average-sized metastases – 4 points and multiple average-sized metastases – 6 points; few large metastases were denoted by 3 points, an average number of large metastases – 6 and multiple large metastases by 9 points. We then added together the figures of metastasization thus obtained organ by organ, and deduced the mean indices calculated in terms of one rabbit. In this way we constructed the diagrams which are reproduced below. Our results were treated statistically by the method of probability of the presence of a correlation by calculation of the coefficient of association χ^2. This method serves to determine the adequacy and the reliability of the experimental material.

The second series of experiments was undertaken in order to exclude the possibility of inequality in the accumulation of tumor cells in the normal and collapsed lung. For this purpose we transplanted equal volumes of Brown-Pearce tumor emulsion simultaneously into the right and left lungs, and then induced a unilateral artificial pneumothorax.

We carried out the simultaneous transplantation of Brown-Pearce tumor into the lungs of 20 rabbits, in a dose of 0.3 ml of 20% tumor emulsion in each lung. The transplantation was done as follows: along the course of the seventh rib, in the right and left axillary regions, skin incisions 2 cm long were made. The subcutaneous tissues, the latissinus dorsi and serratus muscles were divided on both sides and the thorax was exposed. Along the outer edge of the longissinus dorsi muscle, in the seventh intercostal space, a needle was introduced on each side to a depth of about 0.4 cm. The tumor suspension of Borwn-Pearce rabbit carcinoma was carefully inoculated beneath the visceral pleura.

An artificial pneumothorax was immediately induced on the right side in 13 rabbits by introduction of 75 cc of air into the pleural cavity. The left lung, with no pneumothorax, acted as a control. The remaining seven rabbits were controls. Five days after induction of the artificial pneumothorax, 75 cc of air was insufflated into the right pleural cavity of the experimental rabbits. Ten days after transplantation of the tumor, both experimental and control animals were sacrificed, autopsied and the lungs carefully examined.

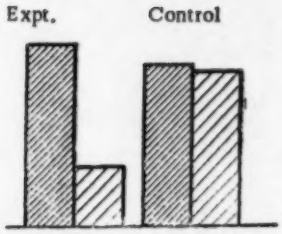

Fig. 3. Degree of metastasization of a Brown-Pearce tumor in the lungs. a) Experiment; b) controls. Closely shaded part – metastasization in the left lung; lightly shaded part – metastasization in the right lung. The columns represent the mean indices of metastasization in the lung for each group and are calculated in terms of one animal.

RESULTS

In the first series of experiments with intratesticular implantation of the Brown-Pearce tumor, the transplantation was successful in 79 rabbits. In two rabbits of the experimental group and one of the control group the tumor did not take. In the experimental group metastasis formation took place in 39 of 40 rabbits, and in the control group, in 35 of 39 rabbits. Differences were observed in the metastasis formation between the experimental and control animals: in the rabbits of the experimental group the degree of metastasization in the organs was less than in the controls (Fig. 1). The difference in metastasization was statistically significant. The value of $\chi^2 = 25.95$ and $\eta = 5$. On the basis of Fisher's χ^2 table, P for these values is considerably smaller than 0.01. Consequently, the change in the functional state of the lung as a result of the induction of an artificial tension pneumothorax brings about a decrease in the degree of metastasization in the organ.

Metastases were present in the lungs in 20 of the 39 rabbits in the experimental group. A distinctive feature of their distribution was that the left lung was more severely affected by metastases (the metastases in the lung were both larger and more numerous) than the right, collapsed lung (Fig. 2). In the control group metastases in the lungs were observed in 16 of 35 rabbits. Their distribution by number and size was the same in both lungs. It will be clear from Fig. 3 that the degree of metastasization in the lung affected by the pneumothorax was only 40% as high as that in the opposite lung. The difference in the degree of metastasization is statistically significant. The value of $\chi^2 = 19.73$, $\eta = 5$. The probability P corresponding to these values is much less than 0.01.

The study of the development of metastases in the lungs when the functional state of one of them is modified by the induction of an artificial tension pneumothorax thus revealed a weaker development of metastases in the collapsed lung.

In the second series of experiments with transplantation of a Brown-Pearce tumor in both lungs, in all 13 experimental animals the development of a tumor was observed in the left lung, whereas in the right lung the tumor completely failed to develop in 4 rabbits, in 7 it was much smaller than in the left, in one rabbit the tumor was the same size in both lungs and only in one rabbit was the tumor in the right lung significantly larger than in the left. In the control animals tumors developed in both lungs and in 6 rabbits they were perfectly identical in size. Only in one case was the tumor in the left lung significantly larger than that in the right. Thus in the overwhelming majority of animals, the tumor developed more rarely in the right lung and grew more slowly than in the left lung, which was not affected by pneumothorax, in spite of the fact that an equal volume of tumor cells was introduced into the two lungs.

It is impossible to explain the weaker development of metastases in the collapsed lung by the possibility of the mechanical effect of tissue compression. Admittedly, it has been considered until recently that the filling of the pleural cavity with air places the lung in a state of compression and completely or partially excludes it from the function of respiration. Modern physiological and clinical findings do not fit in with the old ideas of the biomechanics of the lungs in pneumothorax. Work by several authors [1, 3, 4, 5, 9, 10, 14, 20, 22] has shown that, after the induction of an artificial pneumothorax, the ventilation of the lungs is not diminished but increased and the compressed lung accomplishes considerable respiratory excursions.

G. E. Platonov [12] has shown that after induction of an artificial pneumothorax the cell respiration in the collapsed lung is intensified, with an increase in the catalase, and, to a lesser degree of the peroxidase activity and a moderate increase in glycolysis. The increase in the oxidative processes leads to activation of bacteriolysis and phagocytosis.

From the foregoing remarks we may postulate that unfavorable conditions for the development of metastases and also for developement of the primary tumor are created in the lung when it is in a state of collapse as a result

of the induction of a pneumothorax, in consequence of changes in its biological functions and in its associated metabolic processes. Furthermore, the change in the functional activity of the lung must inevitably lead to some degree of modification of the general metabolism of the body, which in turn may be responsible for the diminution of the degree of metastasization in various organs. This factor may be associated with changes in the resistance to growth of tumors.

These results are evidence of the important role of the functional state of organs in the process of metastasization.

SUMMARY

Studies of the development of metastases in the lungs after changes in the functional state of one lung caused by induction of an artificial pneumothorax, demonstrated a less pronounced development of metastases in the collapsed lung and reduction of metastasization into organs. The data obtained point to the important role played by the functional condition of organs in the process of metastasization.

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REGENERATIVE HYPERTROPHY OF THE LUNGS IN RATS AFTER ONE-STAGE REMOVAL OF THE ENTIRE LEFT LUNG AND THE DIAPHRAGMATIC LOBE OF THE RIGHT LUNG

COMMUNICATION I. CHANGES IN THE NUMBER AND DIMENSIONS OF THE ALVEOLI AND IN THE THICKNESS OF THE INTERALVEOLAR SEPTA

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 100-105, November, 1960
Original article submitted April 11, 1960

During the last 10-15 years both abroad [14, 19] and in the Soviet Union [1, 10, 13] one-stage bilateral operations for removal of up to 50-60% of functioning lung tissue have been successfully performed. It is, therefore, particularly important to investigate the compensatory powers both of the body as a whole and of the residual lung [2, 3, 5, 7, 11, 12, 16]. Insufficient attention has been paid, however, to the study of the compensatory mechanisms brought into play during exclusion of the greater part of the respiratory surface. For instance, contradictory conclusions have been reached regarding the character of the changes in the residual lung.

In the opinion of a number of authorities [9, 17, 20], after extensive resections emphysema develops in the lung, and this gradually leads to functional atrophy of the organ.

On the other hand, investigations by B. G. Gol'dina [6], A. A. Birkun [4] and I. K. Esipova and E. V. Ryzhkov [8] have shown that the compensatory, adaptive changes in the residual lung after resection are expressed morphologically by the opening up of physiological atelectases, the enlargement of the alveoli and hypertrophy of the interalveolar septa, the regeneration of the elastic framework of the alveoli and the formation of new capillaries etc.

Tiemann [21], Cohn [15] and Hilber [18] cite data showing that the formation of new alveoli may occur, and stress the importance of this process in the restoration of the removed lung tissue.

The aim of the present investigation was to investigate the changes in the size of the alveoli and in the functional capacity of the hypertrophied alveoli, i.e., to obtain numerical data relating to the morphological characteristics of the process of regeneration after extensive resections.

METHODS

The experiments were carried out on sexually mature male rats weighing 197-324 g, kept on the usual briquette diet of the institutions of the AMN SSSR. Under ether anesthesia, and observing the rules of asepsis and antisepsis, the diaphragmatic lobe of the lung was exteriorized through an incision in the sixth intercostal space by means of fenestrated ophthalmic forceps. A silk ligature was applied to the hilum of this lobe, and the lobe was excised by cutting through the parenchyma of the lung with scissors. After the muscles and skin had been

TABLE 1

Number of Animals Used at Various Periods after Operation

Group of animals	Period after operation				Total number of animals
	7 days	1 month	3 months	6 months	
	Number of animals used				
Experimental	8	13	10	11	42
Control	9	7	10	11	37

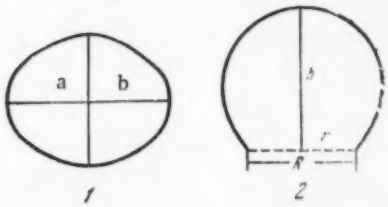

Fig. 1. Types of alveoli measured and their parameters. 1) Alveolus of closed type: a) maximum diameter; b) minimum diameter – a line drawn at right angles to the maximum diameter; 2) alveolus of open type: R) width of inlet to alveolus – a line joining the ends of the open outline; h) depth of the alveolus – a line perpendicularly bisecting R.

closed in layers with silk sutures, an incision was made in the fifth intercostal space on the left, and the left lung excised by the method described above. Disulfan powder was sprinkled inside the chest. Since the left lung accounts for 36.8% by weight, and the diaphragmatic lobe of the right lung 25.7% by weight of the whole lung tissue, in our operations of one-stage removal of the left lung and the diaphragmatic lobe of the right lung, in practice we removed about 63% of the lung tissue. In order to evaluate the results obtained, 79 rats were investigated. The periods of investigation were: 7 days, 1, 3, and 6 months (Table 1).

A comparative appraisal of the state of the lung as a whole (its volume and dimensions) and, in particular, of its morphological structure (dimensions of the alveoli, thickness of the interalveolar septa, etc.) may be obtained only if certain rules of fixation are observed. For this reason the sacrifice of the animals and fixation of the lungs were carried out as follows. Under ether anesthesia the trachea was dissected out and, at the moment of inspiration corresponding to the maximum volume of the lungs, a ligature was applied to it. The chest was then opened with care, in order not to injure the lungs. A 7% glucose solution was then injected under a pressure of 30-35 mm Hg through a cannula introduced through the incision into the right ventricle of the heart and the pulmonary artery, until the bulk of the lung tissue had become pale pink in color, indicating that the lung capillaries were free from blood. The drainage was effected through an incision in the left ventricle. Under the same pressure, Susa's fixing solution was injected into the pulmonary vessels until the tissues had become pale, indicating that the solution had penetrated into the capillaries. The heart-lung tissue-complex was extracted from the chest and immersed in the fixing solution for 24 hours, after which the lungs were treated in the usual way. Before the volume of the lobes was determined the lungs were placed in 70° alcohol until they sank to the bottom of the vessel, indicating that the air had been expelled from most of the alveoli by the alcohol. The volume of the lungs was determined by the volume of 70° alcohol expelled into a graduated cylinder. The upper lobe of the right lung, facing in the same direction in every case, was embedded in paraffin wax and sections of it were cut to a thickness of 7.15μ and 25μ. The sections were stained with hematoxylineosin by Mallory's method and with fuchsin by Weigert's method. To define the histological characteristics of the lungs, ten animals were studied (5 experimental and 5 control) at each period. By means of an Abbe drawing apparatus, under magnification of 400 times, from the sections cut to a thickness of 25μ the outline of 100 alveoli of closed type and 100 alveoli of open type were traced on paper (Fig. 1). By using such a large number of alveoli it is possible to smooth out the differences in their dimensions resulting from differences in the degree of stretching of the alveoli in different areas of the lobe. The area of the closed alveoli was calculated from the fomula for the area of an ellipse ($S = \pi ab$), having first measured the maximum diameter of the alveolus and the line perpendicularly bisecting this diameter. In order to describe the alveoli of open type, two measurements were taken: R – the width of the inlet to the alveolus and h – the depth of the alveolus, i.e., a line perpendicularly bisecting the line closing the inlet to the alveolus (R). The values of these measurements were used to determine the volume of the alveoli from the formula for the volume of a segment

TABLE 2

Changes in the Volume of the Residual Lobes of the Right Lung at Different Periods after Operation

Group of animals	Time of observation	Volume of the left lung (in cc)	Vol. of right lung (in cc)					Vol. inc. related to rt. lung of control animals without diaphragmatic lobe (in %)	Vol. inc. related to both lungs of control animals (%)
			diaphragmatic lobe	upper lobe	middle lobe	accessory lobe	total		
Experimental	7 days	—	—	1,40	1,48	1,32	4,20	124,5	—
Control		1,70	1,35	0,56	0,71	0,60	3,22		
Experimental	1 month	—	—	2,25	2,36	2,32	6,93	219,3	15,5
Control		2,00	1,83	0,65	0,78	0,74	4,00		
Experimental	3months	—	—	2,37	2,35	2,21	6,93	228,4	25,5
Control		1,90	1,51	0,67	0,74	0,70	3,62		
Experimental	6 months	—	—	2,36	2,43	2,52	7,31	293,0	37,6
Control		1,88	1,57	0,55	0,70	0,61	3,43		

TABLE 3

Changes in the Area and Volume of the Alveoli and the Thickness of the Interalveolar Septa and in the Number of Alveoli in the Upper Lobe at Different Periods after Operation

Group of animals	Period of observation	Volume of upper lobe (in cc)	Area of alveolus (in μ^2)	Volume of alveolus (in μ^3)	Number of alveoli in upper lobe	Thickness of of interalveolar septa (in μ)
Experimental	7 days	1,40	3576	140×10^3	10×10^1	7,56
Control		0,56	2435	81×10^3	$6,9\times10^8$	5,89
Experimental	1 month	2,25	6763	322×10^3	$7,1\times10^1$	6,47
Control		0,65	3187	128×10^3	$5,0\times10^8$	5,91
Experimental	3 months	2,37	6373	256×10^3	$9,2\times10^1$	7,41
Control		0,67	2713	80×10^3	$8,3\times10^1$	6,60
Experimental	6 months	2,36	6763	329×10^3	$7,1\times10^1$	7,31
Control		0,55	2303	69×10^3	$7,9\times10^8$	6,08

of the sphere:

$$V = \frac{1}{6}\pi h(h^2 + 3r^2), \text{ where } r = \frac{R}{2} \quad .$$

In order to solve the problem of the number of alveoli, the volume of the lobe was divided by the mean volume of one alveolus. In each case we measured the thickness of 50 interalveolar septa throughout the section from the upper lobe by means of an ocular micrometer.

RESULTS

One week after the operation the residual lobes (upper, middle and accessory) of the right lung had already increased in volume by comparison with the analogous lobes of the lungs of control rats. The degree of enlargement of the lobes varied at different times (Table 2).

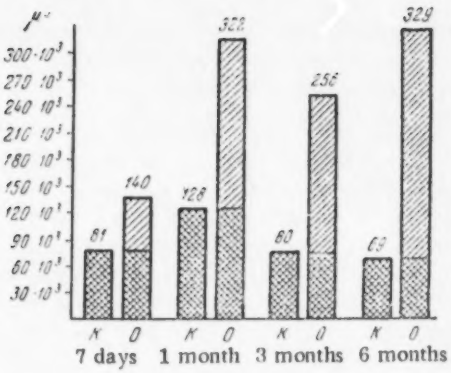

Fig. 2. Changes in the volume of the individual alveoli of the right lung at various periods after operation. K) controls; O) experimental. The more lightly shaded part of the column represents the increase in volume over that of the controls.

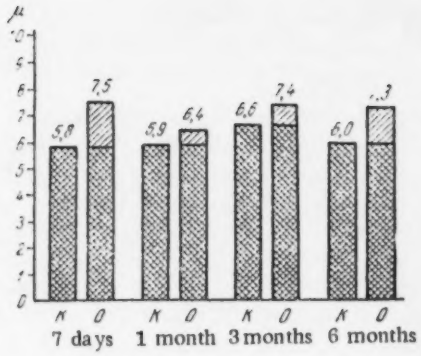

Fig. 3. Thickness of the interalveolar septa of the right lung at various periods after operation. K) controls; O) experimental. The more lightly shaded part of the column represents the increase in thickness over that of the controls.

Seven days later the enlargement of the upper lobe amounted to 150%, the middle lobe 108.4% and the accessory lobe 120% over the volume of the corresponding lobes of the lungs of the control rats. It it is remembered that the total volume of the lobes left behind at operation amounted on the average to 1.87 cc, then the increase in their volume was 2.33 cc (4.20-1.87 cc), i.e., 2.2 times. The volume of the operated lung, however, still had not reached the volume of both lungs of the control animals (Table 2). One month later, the remaining lobes of the right lung were so enlarged that they occupied the whole thoracic cavity, displacing the heart to the left. The operated lung not only had reached the volume of the right lung of the control animals, but was actually 15.5% larger in volume than both lungs together of the control animals. Subsequently (3 and 6 months later) a further increase in volume took place, but this was relatively slight.

It may be postulated that the increase in the volume of the residual part of the organ is the result: 1) of an increase in the volume of the remaining alveoli and of hypertrophy of the interalveolar septa, following cellular hyperplasia and an increase in the number of elastic fibers and capillaries; 2) of the opening up of physiological atelectases and subsequent hypertrophy of both the newly opened and the previously functioning alveoli, with preservation of the normal morphological structure of the interalveolar septa; 3) of mechanical stretching of the alveoli with thinning of the interalveolar septa and emptying of the capillaries, leading to the development of emphysema. Finally, the possibility is not excluded 4) of the formation of new alveoli, the functioning of which may also influence the volume of the operated lung.

Since the increase in the volume of the individual lobes of theremnant of the lung was roughly the same, we shall subsequently use only the results obtained from the study of the upper lobes, assuming that analogous changes took place in the other lobes.

Analysis of the results obtained by measurement of the alveoli showed that at early periods after operation a considerable increase was already taking place in the area and volume of the alveoli (Table 3).

On the basis of these findings and of measurement of the volume of the upper lobe, we provisionally calculated the number of alveoli in the upper lobe.

Seven days later the mean volume of one alveolus was increased 1.7 times. As the postoperative period advanced a further increase in volume was observed: after one month the mean volume of one alveolus was 2.5 times, after 3 months 3.2 times and after 6 months 4.7 times greater than the mean volume of one alveolus of the lungs of the control animals, as is shown graphically in Fig. 2.

Besides the increase in the volume of the alveoli, mention must be made of the hypertrophy, admittedly very slight, of the interalveolar septa (which is illustrated in Fig. 3), which were rich in cells and capillaries.

On examination of the entire lobe areas of emphysematous change in the parenchyma of the lung could be seen, with grossly dilated alveoli, the walls of which were thinned and lacking in capillaries. These areas were mainly found at the periphery of the lobe and occupied a comparatively small space.

The increase in the volume of the residual part of the organ evidently took place not only on account of the true hypertrophy and emphysema of previously functioning alveoli, but also as a result of the development of "new" alveoli. For instance, after seven days, the number of alveoli in the upper lobe of the lung of the experimental animals, according to our calculations, was greater by 3.1×10^6 on the average than the number in the upper lobe of the lung of the control rats. Since the mean volume of the alveolus at this period was 140×10^3, it may be considered that the addition of a number of alveoli in the early periods after operation takes place as a result of the opening of physiological atelectases, with the subsequent hypertrophy of the alveoli, and not on account of the formation of new alveoli, for the dimensions of such newly developing alveoli would be smaller. We cannot categorically deny that the formation of new alveoli is possible.

If this process does take place, however, it cannot be the principal mechanism of regeneration of the injured organ.

It may be concluded from these results that a leading part in the process of recovery after resection of a considerable proportion of the lung tissue is played by the reconstruction of the parenchyma of the residual part of the organ, accompanied by the opening up of physiological atelectases, by an increase in the volume of both the previously functioning alveoli and of those newly opened up, by an increase in the thickness of the interalveolar septa, by cell hyperplasia and by the formation of capillaries. The appearance of foci of emphysema in the operated lung is not a dominant sign of the process of morphological reconstruction.

Because the increase in the volume of the residual lung, when caused by injury, is mainly the result of hypertrophy of the structural units of the organ – the alveoli – and also of proliferation of the cells of the interalveolar septa and of an increase in the number of capillaries, and because it is regenerative in character this process may be described as regenerative hypertrophy, a characteristic form of regeneration of many internal organs.

SUMMARY

In 42 white rats the left lung and the diaphragmatic lobe of the right lung, which represented 63% of the total lung tissue, were removed. Measurement of the thickness of the interalveolar septa of the volume of the alveoli and their number at different periods after the operation showed that in the process of compensatory readjustment of the pulmonary parenchyma the main role belongs to "regenerative hypertrophy," which is characterized by a considerable increase of volume of the remaining part of the lung. The latter is a result of an increase of volume of the already functioning and of the newly opened alveoli, of thickening of the interalveolar septa of hyperplasia of the cellular elements and of the formation of new capillary vessels.

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THE EFFECT OF REPARATIVE REGENERATION OF THE LIVER ON THE MITOTIC ACTIVITY OF THE CORNEAL AND EPIDERMAL EPITHELIUM IN MICE

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 105-108, November, 1960
Original article submitted November 19, 1959

Changes in the intensity of cell proliferation in the tissues arising after trauma are not confined to the site of injury. For instance, after injury to the liver or extirpation of part of the organ, considerable intensification of cell proliferation takes place not only close to the wound surface but also in other lobes of the liver not affected by the injury [1, 7]. If a burn is inflicted on one cornea in rats, changes take place in the number of mitoses in the intact cornea of the opposite eye [6]. The regular changes in mitotic activity in the epidermis of rats arising after infliction of a skin wound are accompanied by changes of a similar nature in the number of mitoses in symmetrically opposite areas of intact epidermis [5]. The regenerative processes arising as a result of trauma and reflected in the mitotic activity of the tissues thus affect the intensity of physiological regeneration of injured tissues. M. A. Vorontsova and L. D. Liozner [2] have developed the view that the recovery processes in reparative regeneration are based on the power of the tissues to undergo physiological regeneration, and stress that very little research has been done into the problem of establishing a relationship between these processes. This makes it difficult to analyze the relationship between the restorative processes in physiological and reparative regeneration. The problem of how the level and character, for example the diurnal rhythm, of mitotic activity varies in different tissues when a focus of reparative regeneration is present in the body is another which has been insufficiently investigated.

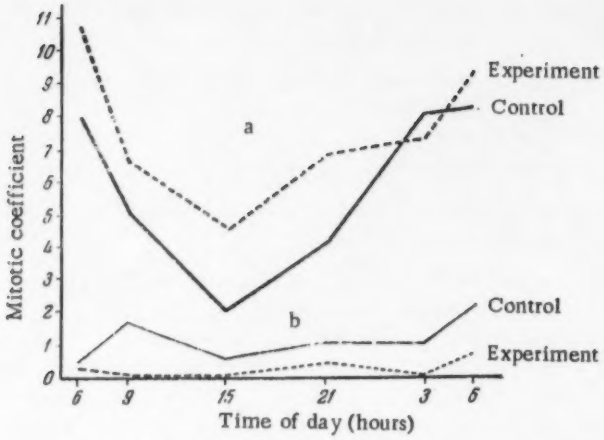
The aim of the present work was to study the changes in the mitotic activity of the epithelium of the cornea and epidermis in mice after extensive injury to the liver.

METHOD

The experiments were conducted on white mice, from which the left lateral and central lobes of the liver (60-70% of the organ) had been removed. The animals were sacrificed by decapitation on the second and third days after operation, at 6 A.M., 9 A.M., 3 P.M., 3 A.M., and 6 A. M. At each of these times 5 experimental and 5 control animals were studied.*

The material for histological examination was fixed in Zenker's fluid. Total preparations were made from the corneas of both eyes, and stained with hematoxylin by Carazzi's method. The concha of the external ear, cut longitudinally from base to apex, was embedded in celloidin-paraffin wax, and sections were cut to a

*The work was carried out on the same animals as were used for the investigations by L. D. Liozner, Z. A. Ryabinina and V. F. Sidorova [7] into the pattern of the mitotic activity of the liver during its reparative regeneration.



Changes in the mitotic coefficient in the corneal epithelium (a) and in the epidermis (b) of the experimental and control mice.

thickness of 8μ. These sections were also stained with Carazzi's hematoxylin. All the sections were examined under an immersion objective 90× and an ocular 7×.

The mitoses in the cornea were counted on both sides of two mutually perpendicular lines drawn on the slide and intersecting at the center of the cornea. In addition to the mitoses in various areas of the cornea, in certain fields of vision of the microscope we counted all the cells in order to calculate their mean number per field of vision. In each cornea the mitoses were counted in 120-140 fields of vision.

The mitoses and cells in the epidermis of the ear were counted on both the external and internal surface of the conchae in 40 fields of vision, on each side, starting from the tip of the auricle. On the average there were 7000-9000 cells in each case.

For both the epidermis and the corneal epithelium we calculated the mitotic coefficient – the number of mitoses found per 1000 cells. The figures obtained were treated statistically by the Fisher-Student method.

RESULTS

Corneal epithelium. The results of the counting of the mitoses in the corneal epithelium of the experimental and control animals are shown graphically in the figure. The curves show that the mitotic activity in the corneal epithelium of the animals of both groups underwent regular changes in the course of the 24 hours. The highest mitotic activity was observed at 6 A.M., after which it gradually fell and, nine hours later, i.e., at 3 P.M., it reached its minimum. This fall in the number of mitoses in the time interval between 6 A.M. and 3 P.M. was statistically significant (for the group of control animals $P = 0.001$ and for the experimental group $P = 0.01$). The mitotic activity then gradually increased. In the group of control animals it reached its maximum at 3 A.M. and remained at this level until 6 A.M. The increase in the mitotic activity in the control animals in the time interval from 3 P.M. to 3 A.M. was statistically significant ($P = 0.004$). In the group of experimental mice the gradual increase in the number of mitoses continued until 6 A.M. ($P = 0.01$).

The figures obtained, showing the diurnal variation in the number of mitoses in the corneal epithelium of mice, were in full agreement with the results of M. T. Gololobova's investigations [3, 4], apart from the fact that in our investigations the minimum number of mitoses occurred at earlier hours than was found in Gololobova's experiments.

Comparison of the course of the curves of variation of mitotic activity in the control and experimental mice shows that the mean values of the mitotic coefficient in the experimental animals at most times of day were higher than in the controls. At 3 P.M. this difference was statistically significant ($P = 0.01$) and at 9 P.M. it was close to significant ($P = 0.05$).

It may hence be concluded that reparative processes in the liver have a stimulating effect on the intensity of physiological regeneration in the corneal epithelium in mice.

Besides counting the mitoses in the cornea of the experimental and control mice, we determined the phases of mitotic division of the cells. We were unable to detect any significant changes in the relative proportions of the individual phases of mitotic division at any time of day. The ratio of early to late phases of mitosis in the control and experimental mice was close to unity.

Epidermis. The changes in mitotic activity found in the epidermis of the external ear in the normal and experimental mice are shown graphically in the figure.

In the first place attention must be drawn to the relatively low level of mitotic activity in this tissue. This factor was obviously the reason why the diurnal rhythm of mitosis in the epidermis was less clearly marked than in the cornea. In the epidermis of normal mice at 6 A.M. a small number of mitoses was found. At 9 A.M., however, their number had risen considerably and had reached its maximum level. Such an increase in mitotic activity during this interval of time was close to significant ($P = 0.02$). The number of mitoses then fell again, reaching its minimum value at 3 P.M. ($P = 0.05$). In the interval between 3 P.M. and 3 A.M., the number of mitoses increased very slowly, but in the interval from 3 A.M. to 6 A.M. the mitotic activity increased considerably ($P = 0.05$). Comparison of the values of the minimum mitotic activity observed at 3 P.M. with the mitotic activity at 6 A.M. next morning shows that this increase was statistically significant ($P = 0.01$).

The figures obtained, showing the change in the number of mitoses in the epidermis over a period of 24 hours, were close to those obtained by M. T. Gololobova [3, 4] in experiments with the epidermis of rats and mice. In her investigations, however, a higher level of mitotic activity of the epidermal cells was found.

The curve reflecting the diurnal variations in the number of mitoses in the epidermis of the experimental mice in its general form repeats the course of the curve of the changes in the number of mitoses in the control animals. Because of the very low values of the mitotic coefficient, however, it is not possible to show statistically whether the differences in the number of mitoses at the different times of day were significant.

During the comparison of the number of mitoses in the epidermis of the control and the experimental mice, attention is primarily drawn to the fact that at all times of the investigation the mean mitotic activity in the experimental animals was lower than the mean mitotic activity in the control mice. These differences, especially in the period of high mitotic activity in the epidermis of the control mice, were statistically significant. For instance, at 9 A.M. $P = 0.001$, at 3 A.M. $P = 0.01$ and at 6 A.M. on the third day $P = 0.01$.

It may be concluded from these findings that extensive injury to the liver and the ensuing repair processes in the organ lead to a lowering of the level of mitotic activity of the epidermal cells of experimental mice.

If the results obtained in this work, on the changes in the mitotic activity of the corneal epithelium and the epidermis of the external ear after the operation of partial hepatectomy, are analyzed it may be concluded that different tissues react differently to injury of the liver: in the cornea of mice an increase in mitotic activity may be observed after operation on the liver, but in the epidermis, on the other hand, depression of mitotic activity takes place. The actual causes of the differences in the response reaction of these tissues cannot yet be established without special investigations.

SUMMARY

The author studied changes in the mitotic activity in the epithelium of the cornea and in the epidermis of the conchae auriculae on the 2nd and 3rd day following reaction of 2/3 of the liver. It was found that the mitotic activity in the epithelium of the cornea in the experimental animals was much higher than in the control group. Diurnal variations in the mitotic activity typical of normal animals was retained. Mitotic activity in the epidermis of the ear in the experimental group of animals was at all times lower, on the average, than in the control group.

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STIMULATION OF THE PROLIFERATION OF THE EPITHELIUM OF THE MAMMARY GLANDS WITH FOLLICULIN IN HYPOPHYSECTOMIZED, OOPHORECTOMIZED MICE

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 109-112, November, 1960
Original article submitted November 16, 1959

The study of the hormonal regulation of the growth of the epithelium of the mammary glands in normal conditions is of great interest to oncology. In the view of the majority of contemporary oncologists and endocrinologists, tumors of the mammary glands arise as a result of pathological changes in the balance of the mammotropic hormones of the pituitary and ovaries [4, 6, 7, 8]. So far, however, there is no sufficiently clear idea of the normal hormonal regulation of growth and involution of the mammary glands. Naturally, it is impossible to understand the essence of the hormonal disturbances leading to the formation of tumors of the mammary gland before the normal hormonal relationships have been discovered.

The view most widely accepted amongst non-Soviet scientists is that of Lyons, who considers that the normal development of the mammary glands requires the combined action of prolactin, growth hormone, folliculin and progesterone. Lyons, however, considers prolactin to be the most important hormone, for without it, in his opinion, the ovarian hormones have no influence on the mammary glands. After administration of prolactin alone to oophorectomized, hypophysectomized rats, Lyons observed growth of the mammary glands [15]. Lyons's findings were confirmed by Ferguson [13] in experiments on C_3H line mice.

Lyons's conclusion that estrogens are of secondary importance in the stimulation of growth of the mammary glands are to some extent contrary to the facts accumulated by oncologists. It has been shown that carcinoma of the mammary glands may be produced by the prolonged administration of estrogens in mice and rats, whereas carcinoma develops spontaneously in these animals extremely rarely [8]. Removal of the ovaries from high-cancer line mice at a certain age prevents the development of carcinoma of the mammary gland in 100% of cases, regardless of the obvious presence of milk factor [1].

Clinical oncologists are well aware that mastopathies and carcinoma of the mammary gland are almost always accompanied by hyperestrinization or disturbance of the rhythm of secretion of estrogenic hormones [6, 7, 9, 10]. Taking into consideration these findings, it is difficult to imagine that estrogens, which may cause pathological hyperplasia of the epithelium of the mammary gland and even malignant growth of the epithelium, have no significant effect on the growth of the mammary glands in normal conditions.

Many workers do not share Lyons's views and cite facts contrary to his scheme [2, 3, 4, 5, 11].

In a previous paper we showed that castration of female mice causes the cessation of the mitotic division of the epithelial cells of the mammary glands, and that the administration of folliculin to such animals causes a considerable increase in mitotic activity, the formation of proliferative epithelial nodules, but not the differentiation of their terminal divisions [3]. The problem of whether folliculin in this case acts directly on the

epithelium of the mammary glands or whether its action is mediated through the pituitary and its hormones has, however, remained unsolved.

The object of the present work was to find out whether folliculin may or may not stimulate the processes of proliferation of the epithelium of the mammary glands in hypophysectomized mice.

METHOD

Experiments were carried out on 102 sexually mature female mice. In the first series of control experiments the animals were hypophysectomized and sacrificed ten days later. Two or three thoracic mammary glands on the right side were taken for histological examination. Besides studying the structure and state of the glands, we determined the mitotic activity of the epithelium of the terminal divisions and ducts. Vaginal smears were taken from the mice during the period of ten days. The absence of an estrus cycle indicated total suppression of the pituitary gland.

In the second series of control experiments, 30 days after castration the mice received 8 daily injections each of 25 i. u. of folliculin in 0.1 ml of peach oil. As our previous experiments showed [3], the greatest increase in mitotic activity in the epithelium of the mammary glands in castrated mice takes place after 8 injections of folliculin. The animals were sacrified on the ninth day. The same glands as in the preceding series of experiments were taken for histological examination and for determination of the mitotic activity.

In the main series of experiments, the ovaries were removed from the animals and, 30 days after this operation, the pituitary was removed. Ten days after hypophysectomy, the mice received subcutaneous injections of 25 i.u. of folliculin in 0.1 ml of peach oil daily for 8 days. Histological examination, determination of the mitotic activity and the statistical treatment of the results were carried out by the methods described previously [2].

Hypophysectomy was performed under sodium amytal anesthesia by Bahner's method [12]. After the operation a considerable proportion of the mice died. Only 25% of the animals survived operation.

After the operation the animals received intraperitoneal injections of 1 ml of 5% glucose and 100 units of penicillin. These injections were repeated on the next two days.

For four days after operation the animals were kept in a cage heated by means of a 25 w electric lamp with a reflector.

Many previous experiments have shown that the subcutaneous injection of peach and apricot oil alone into mice in the doses which we used have no effect on the mitotic activity of the epithelium of the mammary glands [3]. In the present investigation we therefore dispensed with controls in which oil alone was injected.

The criterion of total extirpation of the pituitary in our experiments was the stability of the body weight of the hypophysectomized animals, macroscopic and histological control of the site of the pituitary gland and also the absence of an estrus cycle in the animals of the first series.

RESULTS

Of the 42 animals of the first series, only ten tolerated hypophysectomy relatively well. In mouse No. 8 estrus developed on the fifth day after operation, and at postmortem the presence of one quarter of the anterior lobe of the pituitary was demonstrated. This animal was excluded from the experiment. In the remaining nine mice no remnant of the anterior lobe of the pituitary was found macroscopically or histologically. Vaginal smears showed a state of persistent diestrus. The body weight of the hypophysectomized mice remained stable, whereas the majority of the healthy mice used as controls gained appreciably in weight over the period of ten days.

The results of the first series of experiments showed that the technique used for hypophysectomy gave satisfactory results.

Ten days after hypophysectomy, histological examination of the mammary glands of the animals showed severe atrophy, resembling in its character the atrophy arising immediately after castration. The diameter of the ducts and terminal divisions was greatly diminished by comparison with the normal. The number of terminal

divisions was very small. The epithelial lining of the ducts and alveoli was flattened. The cells were reduced in size. The nuclei of many cells were pyknotic. Ducts were often seen with desquamation of the epithelium. The mitotic activity of the epithelium of the mammary glands fell to zero ten days after hypophysectomy in all the mice except No. 6, but in this animal too the mitotic coefficient was low, amounting to 0,66. The effect of hypophysectomy thus resembles the action of bilateral castration [3] on the mammary glands also by the inhibition of mitotic activity.

The second series of experiments conducted on ten control mice gave results as anticipated. As in the previous investigation [3], the injection of folliculin into castrated mice caused a significant increase in mitotic activity (on the average by 9.8 per thousand). It is interesting to observe that, despite some difference in the conditions of performance of this series of experiments and those of the previous investigation (the age of the animals and the time elapsing after castration), the figures obtained for the mean mitotic activity were very close, amounting to 9.8 and 9.41 mitoses per 1000 cells respectively.

Histological sections of the mammary glands showed the picture of the typical action of folliculin. In the terminal segments and along the course of the ducts proliferative epithelial nodules appeared. The epithelial lining of the ducts became thicker and the lumen of the ducts wider than in the glands of the castrated mice. The formation of terminal segments from the compact proliferative nodules did not take place, as it does in pregnancy.

In the last and main series of experiments, the mice were subjected to bilateral castration followed by hypophysectomy, after which folliculin injections were given. Of the 50 mice undergoing operation, only 13 survived. At postmortem on mouse No. 1 an unexcised portion of the pituitary was found, so that mammary glands were taken from only 12 mice for investigation. Histological examination of the glands showed that administration of folliculin produced a marked reaction of the epithelium in the mammary gland. The character of this reaction was similar to that of the reaction of the mammary glands of castrated females to folliculin. It must be pointed out, however, that the reaction was much weaker in this series of experiments. Although proliferative epithelial nodules developed, they were fewer in number. The thickening of the epithelial lining and the dilatation of the lumen of the ducts were less pronounced than in the castrated animals with intact pituitary gland. The mitotic activity of the epithelium of the mammary glands rose on the average to 4.38 mitoses per 1000 cells, whereas in the control series of experiments it was practically equal to zero. Comparison of the mean values of the mitotic activity in the animals of the second and third series shows that the difference between the results obtained was statistically significant ($P < 0.0001$).

Analysis of the results shows that folliculin, independently of prolactin and growth hormone, in certain conditions may stimulate proliferation of the epitheliumof the mammary glands.

At first sight this conclusion is contrary to the results of the work of Lyons [15] and Ferguson [13]. These contradictions may, however, be readily explained. In his experiments, Ferguson gave injections of estradiol and stilbestrol to hypophysectomized mice for periods of 6 months or more. Such experimental conditions are far from physiological. We have shown that the continuous administration of folliculin to castrated mice first stimulates proliferation of the epithelium of the mammary glands, but that later, starting on the tenth day, this stimulation is greatly weakened [3]. It may naturally be considered that the mammary glands of the mice in Ferguson's experiments were in a state of atrophy at the time of sacrifice, since the stimulating action of the estrogens on growth had long ago ceased.

When he speaks of growth of the mammary glands of mice, Lyons means by this only that lobular and alveolar growth observed in the second half of pregnancy. It is this growth which he attempts to reproduce, choosing for this purpose a combination of pituitary and ovarian hormones [14]. Meanwhile the study of the hormonal regulation of proliferation of the epithelium of the mammary glands in nonpregnant animals and in the first half of pregnancy is of the greatest interest to oncology. At this time proliferation of the undifferentiated epithelium takes place, leading to the formation of compact proliferative epithelial nodules. In the second half of pregnancy growth of the glands and differentiation of the epithelium, typical of the organ in question, takes place. It must not be forgotten that carcinoma of the mammary gland is found more frequently in women who have had few or no children and in women who have not practiced breast-feeding. The second half of pregnancy and especially lactation thus have a normalizing influence on the epithelium of the mammary glands.

SUMMARY

Sexually mature female mice were subjected to hypophysectomy and bilateral ovariectomy. Following this the animals received 25 i.u. of folliculin daily for 8 days and were killed on the 9th day. Mitotic activity of the epithelium in the acinin and ducts of the mammary glands was assessed histologically. In the control series of experiments hypophysectomy was followed by atrophy of the mammary glands and arrest of mitotic activity in the epithelial cells. In hypophysectomized and ovariectomized animals mitotic activity of epithelium of the mammary glands increased on the average, to 4.38 mitoses per 1000 cells after injection of folliculin. However, this rise of the mitotic activity was considerably less than in the control group of castrated animals with intact hypophysis (9.8 mitoses). The above experiments show that in female rats which lack hypophyseal hormone folliculin will definitely stimulate proliferation, of epithelium in the mammary glands, though it fails to produce growth typical of the latter organ.

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FUNCTIONAL AND MORPHOLOGICAL STATE OF THE OVARIES AFTER HYSTERECTOMY

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Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 112-115, November 1960
Original article submitted July 28, 1959

In connection with improvements in surgery, anesthesia and antibiotics, recent years have seen an increasing application of surgical methods to the treatment of certain gynecological diseases. By comparison with the preceding decade, operative treatment of a common condition such as fibromyoma of the uterus has been used twice as often in the past ten years, and by comparison with the decade before than, three times as often as radiotherapy or x-ray therapy. Hysterectomy is the operation usually performed. The question arises, however, of the importance of the uterus to the function of the ovaries and of the body as a whole, and this has received little study. In the Soviet and non-Soviet literature experiments are described, the purpose of which was to show whether hysterectomy has any effect upon the function and morphology of the ovaries. For instance, according to the observations of A. P. Parfenov [5], after removal of the uterine cornua in rats, irregularity of the sex cycle appeared only after three months. After four months estrus developed every 8–10 days instead of every 4-5 days; after ten months and later the intervals between estrus were considerably prolonged and sometimes estrus was absent altogether. Roughly the same results were obtained by N. P. Verkhatskii [1]. According to M. D. Gutner and V. N. Aleksandrovskii [4], during the first few days after operation disturbances take place in the cycle; in the majority of animals it is restored to normal after 18-20 days. A prolongation of the period of estrus or of the resting stage is all that is observed. In some cases estrus disappeared until the tenth week and the intervals between estrus were irregular and prolonged. The observations of L. A. Shusser [6], continuing for a period of 8 months, showed that the intervals between estrus were prolonged on the average by 2-5 days, but apart from that, no abnormality was observed in the sex cycle.

The morphological condition of the ovaries was first studied experimentally in rabbits by I. N. Grammatikati [2]. On the basis of the detection of folliculin in different stages of development, he came to the conclusion that hysterectomy does not affect the ovarian activity. A. P. Parfenov, M. D. Gutner and V. N. Aleksandrovskii [4], N. P. Verkhatskii [1] and others discovered death of the follicles and other changes in the ovaries several months after operation. Intensive development of the corpora lutea was found. Some authors have attempted to explain the development of degenerative processes in the ovaries by disturbance of the anatomical connection between the uterus and ovaries. This point of view is supported by the work of N. P. Verkhatskii [1], M. D. Gutner [3], L. A. Shusser [6] and others.

In view of the discrepancies in the findings so far obtained, we decided to study the functional state and morphology of the ovaries after hysterectomy in our own experimental material.

METHOD

The investigation was conducted on 57 sexually mature female white rats weighing 150-200 g, of which 18 were controls. The functional state of the ovaries was studied by daily examination of vaginal smears. These

were obtained by washing with a sterile pipet, and then treated and stained with hematoxylin-eosin by the usual method. After several weeks of observation, when the regular sequence of the sexual cycles had been established for each rat, operations were performed on the animals. In all 39 rats underwent operation (6 series with 4-8 rats in each) at the same time of year – in the winter of 1956-57. Under ether anesthesia laparotomy was performed. A ligature was applied to the mesentery of the uterine cornua and both cornua completely excised. The stump of the uterus and the vessels were ligated with one or two fine catgut or silk ligatures and the abdomen was closed in layers. Control animals, from which vaginal smears were also taken daily, were kept under identical conditions with those undergoing operation. No smears were taken from the operated animals of each series (nor from the control animals of this series) for one week after operation. The taking of smears was then resumed until the time when the animals were sacrificed. In the course of a year about 10,000 vaginal smears were taken, treated and studied.

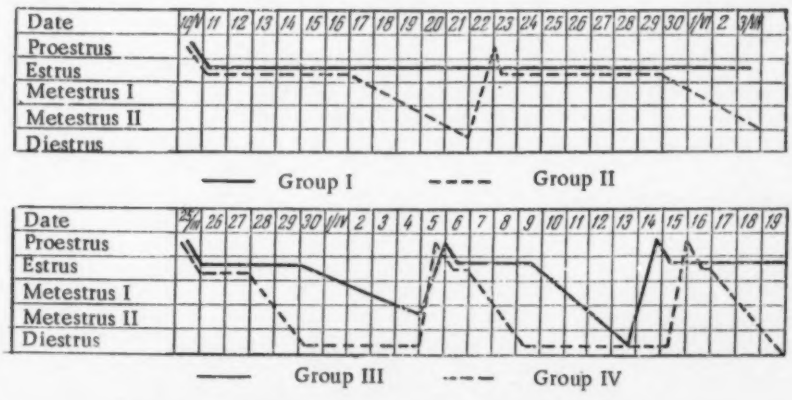
RESULTS

The results of our observations of the sexual cycles are essentially as follows. The sexual cycle in the control animals, and also in the experimental animals showed the following features. The sequence of the sexual cycles at first remained regular. The animals evidently withstood the operative trauma well. Four or five weeks after operation, however, the regular sequence of the sexual cycles was disturbed. The character of the disturbances varied; the experimental animals could be divided in this respect into several groups. The first group (15 rats) showed considerable prolongation of estrus (up to one month); only occasionally could the other stages of the cyle be observed. The second group (11 rats) showed prolongation of estrus to 7-8 days and slight prolongation of the metestrus stage. The third group (7 rats) showed a more or less regular sequence of a cycle which was prolonged to ten days. In this cycle the number of days of estrus varied between three and seven. Subsequently the cycle was shortened to 5-6 days, but at the same time the number of days of metestrus or rest was increased. The fourth group (6 rats) showed a prolongation of the resting stage to 3-6 days.

Typical examples of these cycles are shown graphically in the figure.

It must be pointed out that in certain rats disturbances of the usual pattern of the vaginal smears of different stages of the cycle took place even during early periods after operation. In the literature we were unable to find any description of an atypical pattern of the viginal smears. We frequently observed round, atypical squamous cells, showing basophilic staining properties, confluent round cells, small dark basophilic cells, small cells with round, dark blue nuclei, elongated cells with dark nuclei and mucus and leukocytes, sometimes in large quantities. The variations were not observed in the control animals, so that they could not be attributed to fortuitous causes.

We studied the ovaries of control and operated rats sacrificed 4, 8, and 12 months after operation. Both ovaries appeared identical macroscopically. In the rats sacrificed one year after hysterectomy, the ovaries were considerably reduced in size and had undergone cystic changes. In the ovaries of the rats sacrified 4 months after operation, corpora lutea were much more in evidence than other structures. The number of corpora lutea at



Changes in the sexual cycle of rats after hysterectomy.

different stages of development reached 15-16 in one section. Few follicles were observed at the different stages of development, and the Graafian follicle stage was rarely seen. Many follicles were in a state of atresia. From 8 to 12 months after operation atrophy of the ovaries was observed, and this was particularly marked in cases of cystic change, when the bulk of the ovary was composed of huge cavities, filled with brown contents and bounded by thin walls lined with a flattened epithelium. The atrophic ovaries contained numerous corpora lutea but no primordial follicles, all the follicles present being in a stage of atresia; the Graafian follicle stage was never observed.

Our findings support the view that hysterectomy is soon (after 1-2 months) reflected in the function of the ovaries and disturbs their activity, and a few months after operation morphological changes in the ovaries become evident.

As pointed out above, the disturbance of the activity of the ovaries is primarily expressed by the persistence of estrus. This fact has been observed by many workers, but they have not given any explanation of it. We believe that the cause of the persistence of estrus is the persistence of the corpora lutea and the resulting excess of lutein. Our investigations demonstrated the considerable persistence of the corpora lutea. Meanwhile the Graafian follicle stage is hardly ever seen and atresia of the follicles is found. Loeb [7] has also pointed out that the prolonged existence of the corpora lutea prevents the development of follicles and ovulation. K. P. Ulezko-Stroganova [5] observed that excessive luterinization is accompanied by death of the follicles. The cause of the proliferation of the corpora lutea is not yet clear. Lack of estrogenic hormone, combined with a temporary excess of lutein, is probably characteristic of the disturbed activity of the ovaries. After hysterectomy the overies are evidently deprived of its regulating influence. Hormone secretion continues, but the regular pattern of their production is disturbed.

SUMMARY

Hysterectomy provokes considerable changes of ovarian function in rats. This is primarily manifested in disturbance of the regularity of the sexual cycles. Persistence of rut is especially characteristic; it is evidently explained by a temporary excess of lutein, associated with a gradually increasing deficiency of the estrogenic hormone. Changes in the other stages of the sexual cycle are observed later with prolongation of the intervals between the rut periods. The ovaries continue to function.

Hysterectomy leads to pronounced degenerative changes in the ovaries as well (atrophy, prevalence of atretic follicles, disappearance of primordial follicles).

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THE EXISTENCE OF COLLASTIN AND COLLACIN

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 116-120, November, 1960
Original article submitted December 22, 1959.

Biochemical and electron microscopic researches have considerably broadened our knowledge of the structure of fibrous structures. It has been discovered that an elastic fiber consists of subfibrils, existing in different combinations and embedded in a specific ground substance, the matrix [9, 13]. The subfibrils and matrix are built up of similar protein components, but acid mucopolysaccharides also enter into the composition of the matrix. Enzymes are present in the body which act upon the matrix membrane and on the fibrillary ground substance [13]. In degenerating elastic tissue Findlay [11] and Braun-Falco [7] found mucopolysaccharides, which is evidence of breakdown of the complex structures of the matrix and subfibrils.

Many problems of the pathology of fibrous structures remain unresolved. For example, the origin of the elastic-like material accumulating with age in the skin of exposed parts of the body is uncertain. Similar changes are encountered in certain other conditions. Since the time of Schmidt [16], some authors have regarded this material as elastic tissue [5, 6, 11], which is apparently confirmed by histochemical and physical investigations [7]. Many authors in the past and at the present time, however, cite no less reliable evidence that this material is a derivative of collagen [3, 13, 15]. Difficulties in the definition of elastin and collagen have led to the creation of the hypothesis that elastin may be synthesized from degradation products of collagen and that intermediate products may be formed in the tissues in the presence of disturbances of the enzyme system regulating the different aspects of fibrillogenesis and fibrillolysis which take place in certain pathological states [13].

Much of this problem might have been explained by Unna's view [18] concerning the impregnation of collagen with elastin, if they had been confirmed. In a series of skin diseases and in "senile elastosis" Unna found a modified basophilic elastic tissue, known as elacin. In these conditions the fibers became separated, swelled and sometimes broke up into droplets of clumps of matter. The degenerating collagen fibers, often breaking up into clumps, were covered by normally staining and basophilic elastic and were coverted into collastin and collacin respectively. Elastin may readily be precipitated on the collagen fibers, but a more intimate bond may be formed between these substances – the combination of molecules on the surface of the collagen. More rarely these two substances merge and mingle with each other to form a "colloid". Unna further distinguished the conversion of collagen fibers into basophilic and metabasophilic collagen (without combination with elastin).

Unna had followers, but, in the words of Sternberg [17], his ideas did not evoke a wide response, although they appeared credible. Carol (8) considered that collacin is actually basophilic collagen. If elacin merges with collagen, a homogeneous mixture, or pseudocolloid is formed. Awoki [6] writes that it is not yet known whether impregnation of collagen with elastin can take place. Hass [14], in extensive survey dealing with elastic tissue, does not fully explain Unna's ideas and states that the meaning of the changes described is unknown. A. V. Rusakov [5] does not agree with Unna's interpretation of the histological findings. Nevertheless Unna is almost the only one of the older investigators whose work is mentioned at all in modern histochemical researches [3, 13].

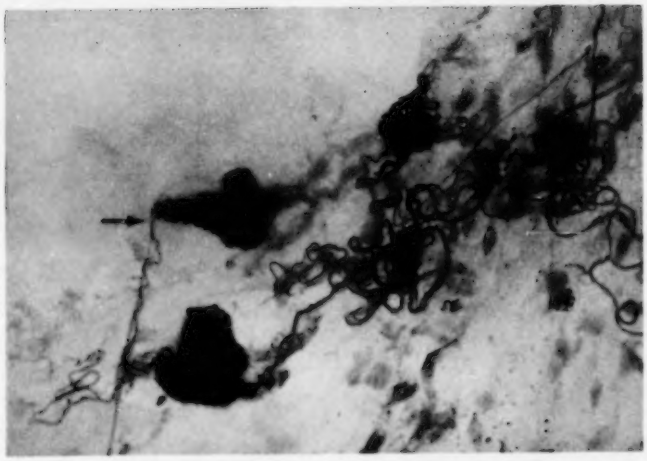

Fig. 1. Film of human loose connective tissue. Lumps of elastic tissue and, adjacent to them, elastic fibrils, here and there entwined to form tufts. The arrow points to a lump in which an area of translucency may be seen, corresponding to a collagen fiber; around it wind subfibrils. Preparation from a woman aged 23 years, dying from chronic rheumatism and heart disease. Fuchselin. Magnification 280×.

When we study films of the loose connective tissue of man and animals we are confronted by the phenomenon of the envelopment of the collagen fibers with modified elastin. The object of the present investigation was to elucidate this phenomenon.

Films were taken from the leg, the abdomen and the arm in human subjects and from various positions in animals, and stained with hematoxylin, picrofuchsin and fuchselin in various combinations, and also with polychrome methylene blue, blue polychrome, azure II, followed by subsequent treatment with a mixture of tannin and fuchsin, and with azure II-eosin.

Elastin derived from localized swellings of elastic fibers, the so-called lumps, was deposited on collagen fibers. We observed the formation of lumps in more than one third of cases (about 30 cadavers of persons aged from 23 to 75 years, dying from leukemia, tumors, contracted kidney and other diseases). Of 11 monkeys (Marcus rhesus) aged 1-2 years, sacrificed for preparation of poliomyelitis vaccine, half of which had suffered from dysentery or pneumonia, they were found in two. The lumps were particularly numerous in the connective tissue of a three year-old monkey which had died from dysentery. Lumps were found in a four year-old rabbit but were absent in a two year-old rabbit. in five dogs and in one sheep. No diseases were noted in these animals.

The lumps were distributed irregularly, in some films as many as 30-35 being present in one field of vision of the microscope with magnification 56× (diameter of the field of vision 2 mm), whereas in others only one or two could be found. The more films were studied the greater was the possiblity of finding swelling of elastic tissue in a particular individual.

The lumps arose as thickenings of the fibers, most of them consisting of bundles of fine companion fibrils and a homogeneous part, forming the groundwork of the swelling (Figs. 1 and 3, 1). Some lumps stained readily with fuchselin, just as well as the adjacent segments of the fiber from which they arose, whereas others were only weakly detected by this stain, and their homogeneous part (matrix) was almost indistinguishable from the background of the preparation, although their subfibrils were easily seen. These lumps gave off only fragments of fibers, which had lost their homogeneity and appeared as bundles of fibrils, continuous with the subfibrils of the lump. Since the formation of lumps has been regarded since the time of Schmidt's [16] research as degeneration of the fibers [6, 10, 18], but in our preparations they were found in sick monkeys* and in human patients (mainly

* We explained the swellings in the rabbit by the animal's age.

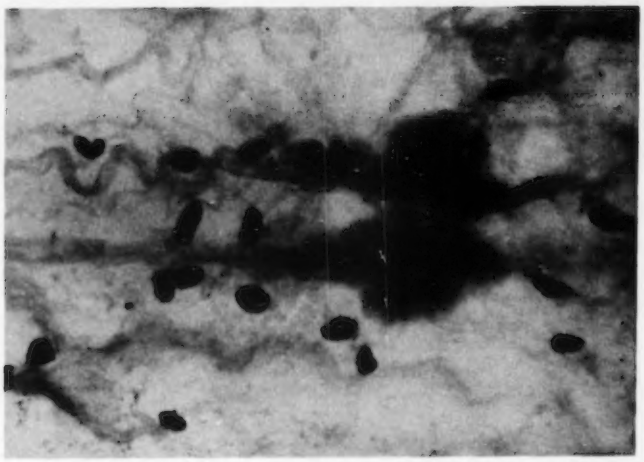

Fig. 2. Film of loose connective tissue (Macacus rhesus, aged 3 years). Basophilic homogeneous lumps. In the lumps the outlines of collagen rods can be seen. Azure II – eosin. Magnification 280×.

elderly), we understood the trend of the process to be destruction of the elastic fibers with gradual lysis of the lumps. Since, however, the lumps remained in the tissues longer than the parts of the fiber not undergoing this metamorphosis, they evidently reflected a delayed (modified) form of elastolysis.

The swellings had a tendency to be situated on collagen and elastic fibers. They could be attached to one side of the fibers, but usually they enveloped them in the form of a cuff, although in this case a very typical appearance was for this cuff to be more pronounced on one side. Staining with fuchselin showed how the subfibrils formed haphazard plexuses and entwined around the collagen fiber (Fig. 1, Fig. 3, 2-5, 8). Combinations of fuchselin with hematoxylin and picrofuchsin clearly showed up the collagen fibers but the component parts of the lumps often stained a uniform black shade. Blue polychrome and other basic aniline stains revealed the lumps (matrix) in the form of dark blue drops and cylinders, having the typical shape of the lumps or more flattened and elongated in shape, or of dark blue deposits on the collagen fibers (Fig. 3, 10-14). In this way the later stages of development of the lumps, not detected by specific staining for elastic tissue, were revealed. Voronin's hematoxylin (with ferric chloride) revealed all stages of conversion of the elastic tissue. Staining with Yasvoin' hematoxylin-picrofuchsin, in the latest stages the lumps acquired a yellowish-brown or reddish color, suggesting their relative oxyphilic properties. Such lumps could be regarded as thickenings of the collagen fibers.

Periodate–fuchsin sulfuric acid stained the swellings a red color. In view of Findlay's results [11], it may be considered that what is actually taking place in the lumps is a breakdown of the complex structure of the subfibrils and of the specific ground substance containing carbohydrates.

Staining with azure II–eosin sometimes revealed a large number of lumps, ranging from dark to light blue. Most were connected with collagen fibers. The thicker fibers were the result of the long, straight or curved shape of the drops of derivatives of the elastic substance. The thinner fibers, however, were associated with small, wedge-shaped protrusions of lumps. Some fibers within the lumps were curved in shape. The elastin drops evidently supported the affected area of the fiber and prevented it from becoming straightened during stretching of the film, and, perhaps, by retaining some degree of elasticity, returning the segment of the fiber to its original state after stretching. The surface of the collagen rods within the lumps and near to them, or less often those visibly unconnected with the lumps, often were stained a violet color with azure, which could be seen in the films as lines or interrrupted marks along the edges of the fibers (Fig. 2, Fig. 3, 12). Hematoxylin also occasionally outlined the edges in this way (Fig. 3, 6). It is possible that some special substance was formed at the point where the modified elastin merged with the collagen. This was in agreement with Unna's statement [18] that the

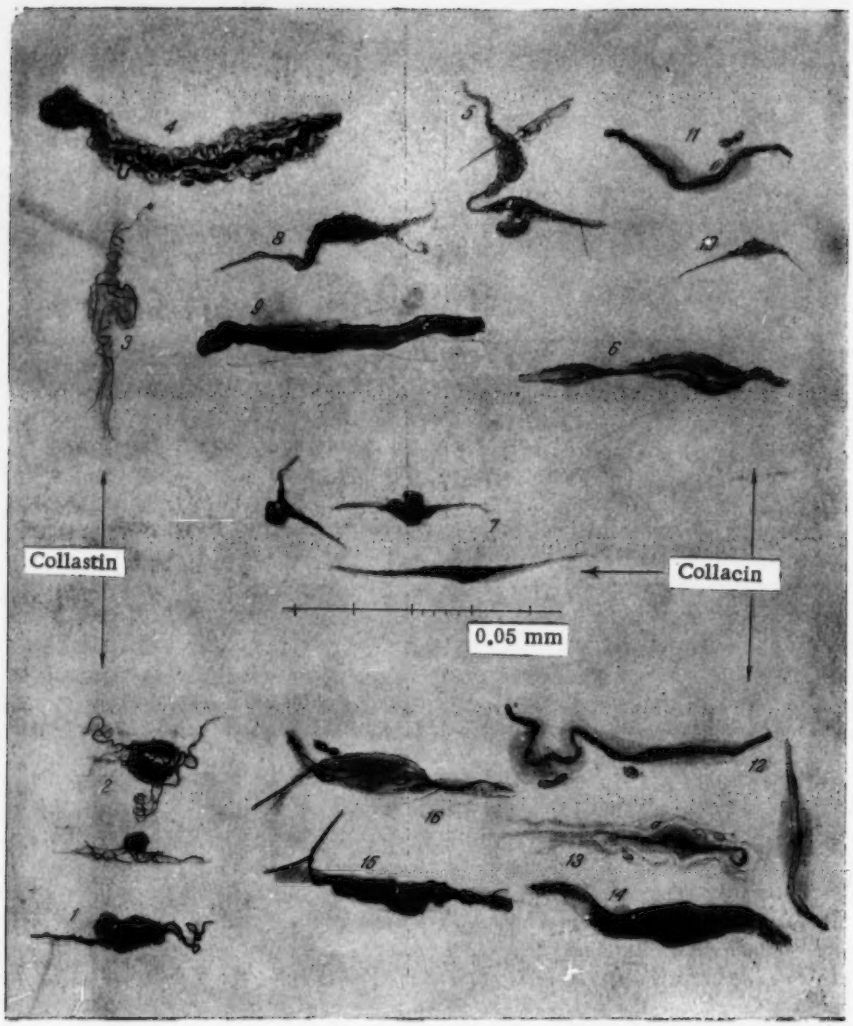

Fig. 3. Semdiagrammatic representation of the combination of elastin with collagen, reproduced by means of a drawing apparatus. Material obtained: 1, 2, 12, 13, 14, 15, 16) from human subjects; 7) from a rabbit; 3, 4, 5, 6, 8, 9, 10) from the monkey. Staining: 1-3) with fucheslin; 4-8) with fuchselin, Ehrlich's or Yasvoin's hematoxylin, picrofuchsin; 9) hemtoxylin with ferric chloride and picrofuchsin (the collagen fiber is covered with a basophilic layer, and palely stained elastic subfibrils can also be seen); 10-12) azure II–eosin; 13-14) polychrome methylene blue, tannin and fuchsin (13–between the lumps and the adjacent collagen fibers is a translucency, probably the result of shrinkage); 15-16) fuchselin polychrome blue, tannin with fuchsin (15–homogeneous staining with fuchselin; 16–black subfibrils can be seen in the blue lump). For explanation see text.

molecules of the two substances were possibly combined.

In certain lumps the collagen rods were much thicker than their continuations outside the swellings. It is probable that the elastin masses, soluble with difficulty and enveloping the collagen fibers, retarded their lysis and thereby made its course irregular.

So far as changes in the collagen are concerned, we could only note that during staining with polychrome blue– tannin the fibrils of some (rare) bundles gave metachromatic staining. Sometimes such changes affected short segments in the whole thickness of the fiber. The tendency of the modified elastin to be situated on the fibrous structures may be explained by its physical properties – by the viscosity of the modified elastin. The property of elastic tissue to adhere, known since last century [1], may also be extended to collagen.

The envelopment of the collagen fibers with elastin at the stage when it stained with fuchselin corresponds to the concept of collastin, whereas their envelopment with basophilic masses corresponds to Unna's concept of collacin. Whereas the extreme stages may be strictly demarcated, however, in the intermediate stages staining with fuchselin is possible, although more weakly, as well as with basic dyes. The simultaneous staining of the subfibrils of the lump with fuchselin and of the homogeneous matrix with, for example, blue polychrome, is not, however always possible (Fig. 3, 16). Unna further pointed out that there was competition between the specific and basophilic stains. This worker doubted whether collacin must pass through a stage of collastin. It may be concluded from our investigations that this occurs very often, if not always.

The presence of small twisted fibrils and of "split" fibers in foci of degeneration of elastic tissue has been observed by many authors, but the interpretation of these changes has been made difficult by lack of knowledge of the structure of the fiber [2, 14]. M. M. Kuznets [2], for instance, describes collacin in the form of slightly basophilic collagen fibers, impregnated transversely and obliquely with very thin violet fibrils, which correspond most of all to elacin. From the study of films in which the tissue relationships are more primitive, and the changes therefore easier to explain, we are satisfied that the thin, twisted fibrils which are often observed in foci of "basophilic degeneration of the skin" are subfibrils.

From the results obtained certain suggestions regarding the changes in the skin may be made. The compact texture of the dermis must account for the more uniform distribution of the degenerating elastin, for the round lumps cannot move amid the intertwined collagen fibers. In certain diseases, moreover, the swellings of the elastic tissue may be more diffuse and their absorption even slower as a result of disturbances of the enzyme systems [13], of a decrease in the sensitivity of elastin to the action of elastase [12] and of the lesser degree of accessiblity of the fibers of the dermis to enzymes in general, by comparison with the fibers of loose connective tissue.

The fact that carbohydrates are found in degenerating elastic tissue in chronic pathological conditions such as senile elastosis, elastic pseudoxanthoma and colloid degeneration of the skin [7, 11] and in vessels of changeable caliber [4], taken together with our findings, suggests that the mucopolysaccharides causing the basophilia of pathological elastic tissue may be detected when destruction of the elastic tissue is retarded and that the discovery of elacin may indicate an abnormality of elastolysis, consisting of delayed lysis of elastomycin.

While, therefore not denying changes in collagen, including those of elastic type, we must support the correctness of the view that collagen is regularly enveloped with modified elastin, a process which occurs when elastolysis is retarded.

SUMMARY

The author studied film preparations of the loose connective tissue in man and animals suffering from various diseases. In many cases focal swellings of the lumps were detected. Their numbers increased with the age of the individual and had less to do with the disease itself. Homogenous basophile matrix part and entangled subfibrils could be identified in the lumps. The lumps had a tendency to place themselves on the collagenous fibers. In earlier stages of their evolution, fuchselin showed the presence of entangled fine elastic subfibrils around the collagenous fibers, which corresponds to Unna's conception of collastin. In later stages of evolution, only the homogeneous basophile masses of elacin are seen around the fibers. These pictures correspond to the conception of collacin. The fact of regular enveloping of fibers with dissolving elastin must be taken into account when studying the morphology of senile elastosis and certain other diseases.

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SCAR FORMATION IN THE CENTRAL NERVOUS SYSTEM UNDER THE INFLUENCE OF PYROGENAL

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 121-123, November, 1960

In the modern view, the principal factor preventing the penetration of nerve fibers through the area of a section of the spinal cord is the rapid formation of scar tissue in the cord. The use of a bacterial polysaccharide enabled the American workers Clemente and Windle [3] to obtain a more friable scar and to observe the penetration of intraspinal nerve fibers through it. Other workers, however using the same preparation, obtained negative results [2, 4].

In view of the great importance of this problem, we also made an attempt to prevent the formation of a dense scar between the cut ends of the transected spinal cord in dogs and also to investigate the possible regeneration of the nerve fibers. As a substance to inhibit scar formation we used the Soviet preparation pyrogenal – a complex polysaccharide of baterial origin, prepared in the N. F. Gamaleya Institute of Epidemiology and Microbiology of the AMN SSSR.

In the present communication we describe the results showing the influence of pyrogenal on the formation of scar tissue.

METHOD

Experiments were carried out on 21 dogs aged from 1 to 2 years. Twelve were experimental animals and nine controls. The spinal cord was transected in the region of the 8th-10th thoracic segment. A longitudinal incision was made in the dura mater, the cord was lifted out on a hook and divided with a fine, pointed scalpel so that the hook was lifted out through the cut. The ends of the spinal cord were placed together and its surface covered with a fibrin film. Pyrogenal was administered systematically at intervals from the second day until nine months after operation, in a dose of 5-10 μg/kg. The animals were sacrificed 14 days and 1, 3, 6, 13 and 26 months from the beginning of the experiment. The spinal cord in all these cases was examined histologically. Serial sections were cut from the area of transection of the cord in a dorso-ventral direction. The sections were stained with hematoxylin–eosin, hematoxylin-picrofuchsin, Spielmeyer's hematoxylin and thionin, and were also impregnated with silver on the argyrophilic fibers of the scar by Foot's method and on the glia by the methods of Cajal and Stern-Beletskii. The nerve fibers were also impregnated with silver by different methods.

RESULTS

Two weeks after operation we observed no marked differences in the structure of the scar tissue in the experimental and control dogs. In the first of these the defect in the cord was replaced by loose connective tissue containing thin, isolated bands of collagen fibers. The wide intervals between the bands were filled with fibroblasts of various degrees of differentiation, histiocytes and numerous macrophages. The scar contained many thin-walled vessels. The scar in the control dogs showed basically the same structural features, but the bands of collagen fibers in the controls were somewhat wider.

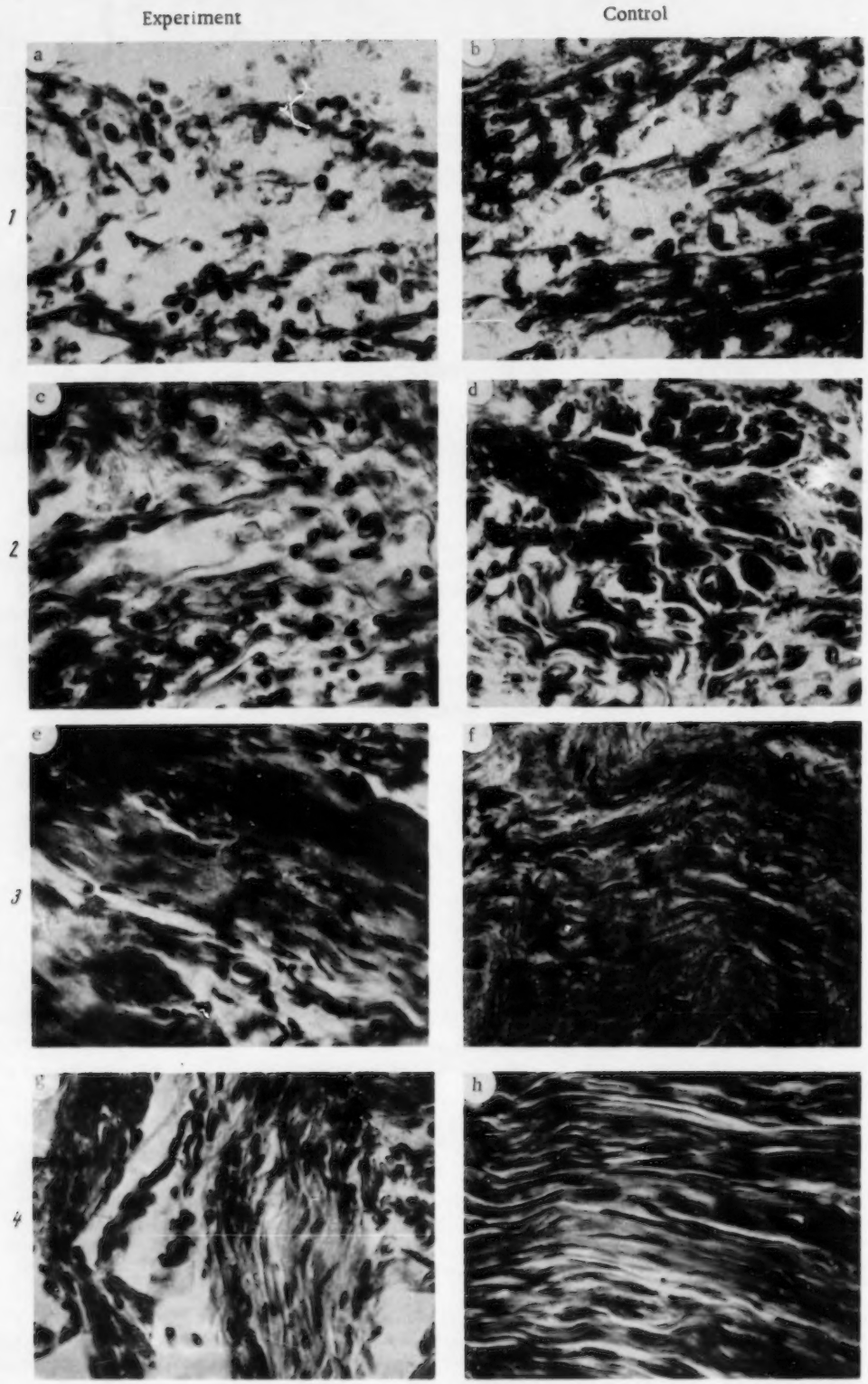

Fig. 1-4. Structure of the scar tissue at the site of transection of the spinal cord in experimental and control dogs. Magnification 400 : 1. Stained with hematoxylin-picrofuchsin and hematoxylin–eosin. 1) 1 month after operation; 2) 3 months after operation; 3) 13 months after operation; 4) 26 months after operation.

One month after operation the character of the scar in the experimental animal had undergone little change (Fig. 1a) and the structural signs found in the scar of the dog examined two weeks after operation were largely preserved. As in the latter case the thin bands of collagen fibers were separated by wide zones of cells, containing as before large numbers of macrophages. The scar contained a fairly dense network of thin-walled vessels.

In contrast to the experimental dog, in the control animal one month after operation the process of scar formation had been carried much further (Fig. 1b). For instance, the bands of collagen fibers had become thicker and were separated by correspondingly narrower zones of cells.

The difference in the tempo of formation of the scar structure was even more obvious at the later periods of observation. Three months after operation the structure of the scar differed sharply in the experimental and control dogs. In the first (Fig. 2c) a slowing of the development of organization of the cord defect was clearly found. Although the number and thickness of the bundles of collagen fibers were slightly increased, the intervals between them remained wide and were filled with connective tissue cells, among which were many macrophages. Considerable numbers of thin- and thick-walled vessels were found in the scar.

The scar formed at this time in the control animal (Fig. 2d) consisted of thick, spirally twisted and haphazardly interwoven bands of collagen fibers. In contrast to the scar in the experimental dog, in which the collagen fibers stained a pink color with picrofuchsin, in the scar in the control dog they were bright red in color. In places very narrow zones of loose connective tissue were found, in which macrophages were absent. Few blood vessels were present in the scar.

Slowing of the tempo of organization of the scar in the experimental animals was also observed at later periods after operation. This could clearly be observed 6, 13, and 26 months after operation (Figs. 3e, and 4g). In the scar in these animals relatively wide zones of loose connective tissue were seen, containing fibroblasts in various stages of differentiation, histiocytes and always a few macrophages. In every case in which pyrogenal was given, thin- and thick-walled blood vessels passed in the loose tissue zones among the bundles of collagen fibers and along the bundles themselves.

In the control animals at these periods (Figs. 3f and 4h) the scar had become even poorer in cells, the intermediate areas of loose connective tissue had disappeared, the bundles of collagen fibers had become adherent to each other and the nuclei of the fibroblasts had acquired an extremely flattened shape. Vessels had almost completely disappeared from the scar, and only a few thick-walled vessels remained near the meninges. In the last case to be observed, signs of hyalinosis of the scar could be observed here and there.

Having noted the well marked influence of pyrogenal on the formation of the structure of the scar, attention must be drawn to the fact that this influence mainly extended to the central area of the scar and had little effect on its peripheral part, in immediate contact with the meninges.

It is thus clear from the pathomorphological findings described above that the administration of pyrogenal influences scar tissue formation in the region of transection of the spinal cord in dogs. In all the animals receiving pyrogenal, at both early and later periods, even after 26 months, we could observe a looser structure of the scar, delay in the maturation of fibroblasts, the presence of macrophages, and better vascularization of the scar tissue. The action of pyrogenal was demonstrated especially clearly by depression of the formation of collagen fibers.

Our findings are in agreement with the observations of Clemente and Windle [3] on the influence of pyromen on the development of a scar in brain tissue. The delayed formation of such a scar facilitates the penetration of nerve fibers along the vessels and the collagen fibers proceeding along the long axis of the spinal cord [1, 5].

We consider that the early use of pyrogenal in the case of injuries to brain tissue may be of practical value in clinical practice, by preventing the rapid formation of a dense scar, with its attendant serious complications.

SUMMARY

Pyrogenal was used in 21 dogs in an attempt to prevent rapid scar formation between the stumps of the severed spinal cord. Pathomorphological studies of the medullary scar were undertaken 14 days, 1, 3, 6, 12, and 26 months after the operation. It was shown that pyrogenal interferes with scar formation at the site of section of the spinal cord. In all cases, even at later dates, the scar of the experimental animals was of a looser structure, the maturation of the fibroblasts was delayed, macrophages were present and the tissues were better vascularized.

Pyrogenal had a more marked effect on the inhibition of formation of collagen fibers.

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WALLERIAN DEGENRATION OF NERVE FIBERS AFTER REPEATED TRAUMA TO NERVE TRUNKS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 123-129, November, 1960
Original article submitted October 21, 1959

The study of the pattern of Wallerian degeneration of nerve fibers arising after division of or injury to nerve trunks is of essential importance to the understanding of the general course of the repair processes taking place in the injured nerve. Many authors [2, 3, 4, 6, 16] have repeatedly drawn attention to the importance of the degenerating peripheral segment of the nerve in its subsequent regeneration. The views of these authors may be summarized by saying that without the preliminary degeneration of the nerve elements there can be no successful regeneration, and that regeneration is possible only in the presence of a degenerated peripheral segment.

As the researches of many authors have shown, the rate of progress of dystrophic processes in the peripheral segment of an injured nerve is not constant, but is subjected to many variations depending on the character of the nerve and the functional importance of its component nerve fibers [9, 10], the species and age of the animal [11, 14], the conditions of nutrition [12] and so on. In cold-blooded animals the time of year and the temperature of the external environment have a definite influence on the velocity of this process. Differences in the rate of progress of the degenerative process in various parts of the peripheral segment of the nerve must also be emphasized [7, 8, 13, 14]. Destruction of the medullary sheaths and the neurofibrillary structures arises first of all in the terminal divisions of the nerve fibers and in the nerve endings, from whence the process spreads to the center, i.e., to the site of injury. There are reports in the literature that the velocity of the progress of Wallerian degeneration may be severely retarded by repeated injury to the regenerating nerve [1].

In view of the practical and theoretical importance of this problem, we decided to study the influence of repeated transection of a nerve on the process of Wallerian degeneration, using both morphological and physiological methods of investigation for this purpose.

METHOD

Experiments were conducted on 12 rabbits. The operation consisted of division of the tibial part of the sciatic nerve with fine pointed scissors at or above midthigh level, at the level of the tendon of the obturator internus muscle. The peroneal nerve was not injured, but acted as a bridge which prevented the separation of the ends of the divided tibial nerve. Form 2 to 2 ½ months later the same nerve was again divided, this time 2 cm above the first scar in six rabbits and 2 cm below the site of the first operation in the other six rabbits. The animals were sacrificed 6, 8, 14, 20, 30 and 50 days after the second operation. The excitability of the divided nerve was first determined in all the animals. As controls, observations were made on the tibial nerve divided once only in 12 rabbits sacrificed at the same times as the experimental animals. Furthermore, in a proportion of the experimental animals the left tibial nerve was divided at the same level at the same time as the second operation was performed on the right tibial nerve. This nerve was also used for control purposes.

The sciatic nerves were fixed in 20% neutral formalin and then investigated throughout the entire length.

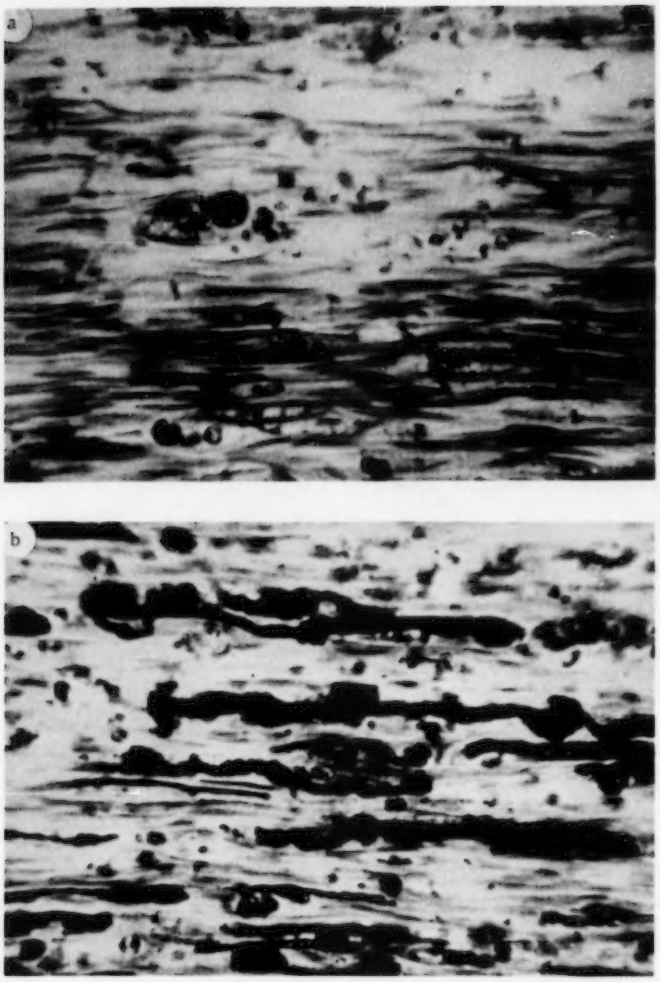

Fig.1. Wallerian degeneration after repeated division (above the site of the initial defect) of a regenerated nerve: a) Distal segment (below the region of the first scar) on the 14th day after operation. The degenerated nerve fibers have been converted into thin bands of Schwann cells, almost free from products of disintegration; b) proximal segment of the nerve (above the site of the first scar) on the 14th day after operation. Large fragments of axis cylinders are seen. Rabbit No. 275. Silver impregnation. Objective 100×, immersion; ocular 2 ×.

Frozen sections were impregnated with silver by the Bielschowsky-Gros method and counterstained with hematoxylin. Some sections of each nerve stained by Spielmeyer's method, with Sudan III and with Sudan black.

RESULTS

The study of Wallerian degeneration after repeated trauma to the regenerated nerve revealed serval distinctive features in the course of this process by comparison with degeneration of the nerve fibers after a single division of the nerve. The distinctions consisted mainly of differences in the rate of the process of disintegration

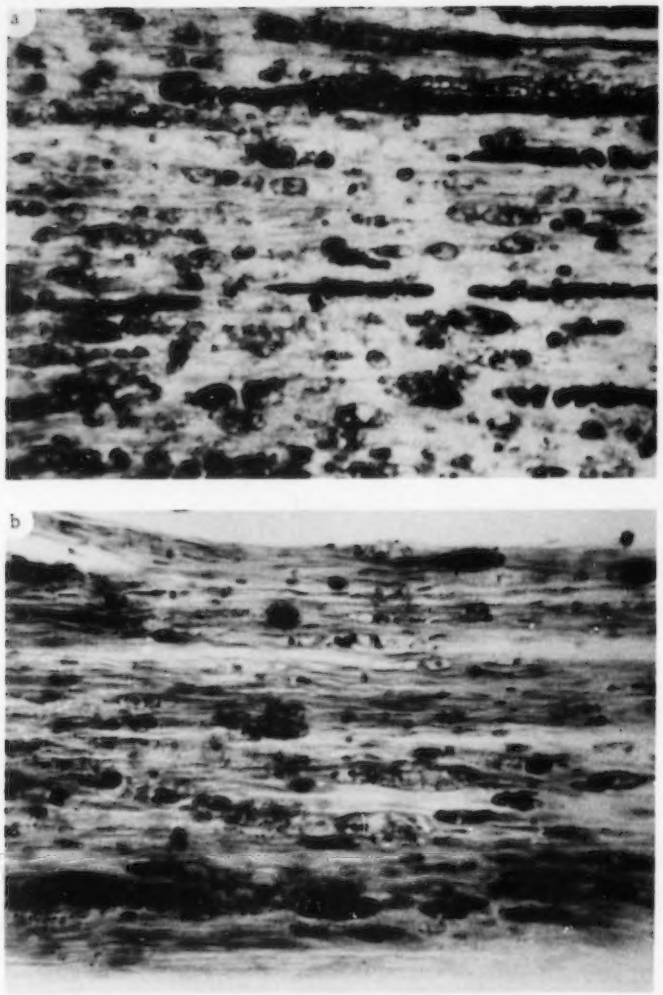

Fig. 2. Degenerative changes in nerve fibers after repeated trauma of the regenerated nerve (division above the site of the original defect) a) Proximal segment of the nerve (above the first scar). In places fragments of medullary sheaths and ovoids, preserving their cellular structure, may be seen; b) distal segment of the nerve (below the first scar). Empty Schwann sheaths can be seen. Stained by Spielmeyer's method. Rabbit No. 374. Objective 45×, ocular 2×.

of the nerve structures and of the resorption of the resulting products. This was most obvious in the case of application of the second injury above the site of the first scar. In these cases the nerve fibers undergoing degeneration had a complex and apparently dual structure. Above the first scar the fibers were essentially normal in their caliber and structure. At the level of the first scar they continued as regenerated fibers which had grown through the scar and the entire peripheral segment of the nerve. During the 2-2½ months elapsing since the first operation, these fibers had not attained their normal structure. Their axis cylinders and medullary sheaths were thinner than usual. After redivision of the nerve above the site of the first scar, these and other areas of the nerve fibers underwent degeneration. However, the tempo of this process showed significant differences between the normal and the regenerated parts of the nerve fiber.

On the 6th-8th day after operation, in the segment of the nerve lying above the first scar (i.e., in the interval

between the second and the first scars), fragmentation and disintegration of the axis cylinders and medullary sheaths took place. The fragments of the axis cylinders swelled, lost their fibrillary structure and often were curiously curved. When stained by Spielmeyer's method an enormous number of ovoids and of elongated fragments of medullary sheaths, covered with connecting bands, were observed. Most of these preserved their cellular structure. Only here and there did the disintegrating medullary sheaths show signs of impending fatty degeneration. This pattern of degeneration corresponded completely to that found at the same times in the control animals.

A quite different picture was found in the segment of the nerve lying below the region of the first scar, where the young nerve fibers, newly formed after the first trauma, were subjected to degeneration. On the eighth day after operation, neither long fragments of the myelin sheaths nor typical ovoids were seen here. By this time they had undergone complete disintegration. The products of disintegration of the axis cylinders had almost entirely disappeared. The degenerated nerve fibers were converted into thin bands of Schwann cells, along the course of which small fusiform swellings were present here and there, containing granular disintegration products. When stained with Sudan III the degenerated nerve fibers showed signs of severe fatty degeneration. Fat droplets were found both in the protoplasm of the Schwann cells and in the region of the fusiform swellings, and also in the protoplasm of the macrophages scattered in the stroma of the nerve.

Tests of the excitability of the nerve showed that on the sixth day after the second division of the nerve stimulation with an induction current (above and below the scar) caused no contraction of the muscles, which agreed completely with the morphological picture of degeneration of the nerve revealed at this period of the experiment.

These differences in the tempo of Wallerian degeneration and resorption of the products of disintegration after the first and second division of nerve fibers were also maintained on the 14th day after operation. Irrespective of the level of the second division of the nerve, in its distal area (situated below the level of both scars) processes of resorption of the products of disintegration predominated. In impregnated sections nearly empty Schwann sheaths of denervated nerve fibers could be seen, in the vacuoles of which only occasionally were small black lumps and grains of disintegration visible (Fig. 1a). When stained by Spielmeyer's method the typical ovoids were absent or present in very small numbers. Lightly stained swellings could be seen along the course of the Schwann cell bands, containing pale granules of disintegration. When stained with Sudan black or Sudan III, numerous small droplets of fat were seen in the protoplasm of the Schwann cells and in the macrophages. The study of the process of degeneration in the part of the nerve situated above the first scar (in cases in which the second disivion was carried out ab above the first scar) or in the peripheral segment of the nerve of the control animal showed the presence of numerous, quite large fragments of axis cylinders and medullary sheaths, together with small lumps and granules of disintegrated matter (Fig. 1b). Only in places were areas of empty Schwann sheaths visible, free from disintegration products over a certain distance. When stained with Sudan III or Sudan black, part of the disintegrating medullated fibers showed the picture of severe fatty degeneration, whereas others, consisting of fragments of medullary sheaths, preserving their cellular structure, did not yet give a positive reaction for fat. Thus above the first scar and in the control nerve the process of disintegration of the medullary sheaths 14 days after operation was still far from complete, and the processes of resorption of the disintegration products were at the beginning of their development.

Twenty days after the second division of the nerve above the site of the first scar the processes of Wallerian degeneration had advanced considerably. In the sections impregnated with silver, the differences between the pictures of degeneration of the nerve fibers above and below the first scar were largely obliterated. In both places the appearance was mainly of empty Schwann sheaths, with scattered elongated nuclei. Only occasionally were separate fragments of axis cylinders seen. The process of degeneration of the neurofibrillary structures in the peripheral segment of the nerve in the control animal was in approximately the same state. So far as the medullary sheaths are concerned, their degeneration took place more slowly than that of the axis cylinders; this meant that on the 20th day after operation a difference could be detected in the tempo of this process after division of the nerve twice or once only. When stained by Spielmeyer's method, the segment of the nerve situated above the first scar, and also the peripheral segment of the control nerve still showed the presence of a fairly large number of ovoids and long fragments of myelin sheaths with a well marked cellular structure (Fig. 2a). At this time, below the first scar, where the regenerated nerve fibers undergoing degeneration, disintegration of the myelin sheaths had advanced much further in its development. Typical ovoids and fragments of myelin sheaths were absent here. The degenerated nerve fibers had the appearance of empty Schwann cell bands, along the course o

of which translucent vacuoles and swellings could be seen in places (Fig. 2b).

These differences in the pattern of degeneration of the nerve fibers after repeated or single injury to the nerve subsequently became less and less pronounced, and the degenerated nerve fibers in both cases acquired a similar appearance, being converted into bands of Schwann cells, rich in fat droplets. Resorption of the fat took a long time, and even one month after the second division of the nerve the protoplasm of the Schwann cells contained large numbers of fat droplets.

These investigations showed that the process of Wallerian degeneration after repeated division of a nerve proceeds far more rapidly than after a single division of the nerve. This is shown by the more rapid disintegration of the axis cylinders and medullary sheaths into separate fragments and ovoids and by the more rapid formation of fat from the disintegration products of myelin. The same may be said regarding the resorption of the disintegration products, especially of the fragments of the axis cylinders, which takes place much more rapidly than usual after a second injury. Consequently the degenerating nerve fibers are converted comparatively early into "empty" bands of Schwann cells, containing large numbers of fat droplets. This corresponds to the rapid loss of excitability of the degenerated nerve. This picture of degeneration of the nerve fibers in the distal segment of the nerve takes place in every case, irrespective of where the second injury is applied – below the first scar (i.e., within the limits of the regenerated fibers) or above the site of the first injury (i.e., within the limits of the apparently normal nerve). In the latter case, i.e., after the second division of the nerve above the first scar, the nerve fibers subjected to trauma are complex in structure.

Each fiber consists of two different parts: the normal fiber and its continuation – the fiber which has regenerated after the first division. From a comparison of the pattern of degeneration of the nerve fibers above and below the first scar, it is easy to be convinced that in the first case it is quite indistinguishable from the degeneration of the nerve fibers of the control nerve, whereas the disintegration of the regenerated nerve fibers is effected at a much quicker tempo. In this respect the regenerated nerve fibers are very reminiscent in their properties of the nerve fibers of young animals, degeneration of which takes place much more rapidly than in adult animals [11]. This suggests that regenerated nerve fibers possess less resistance and that their reactivity is increased.

The quicker tempo of disintegration of the regenerated nerve fibers after division is to some extent associated with their structural features. Even two or three months after trauma the regenerated nerve fibers, their axis cylinders and myelin sheaths are far short of their normal size and structure. Their caliber is thinner than usual. In the degeneration of these fibers, less products of disintegration are therefore formed and they are resorbed more quickly. Such a purely mechanical explanation can hardly apply to the phenomena of fatty degeneration of the myelin. The fat appears earlier in the process of disintegration of the myelin sheaths of these fibers than it does in Wallerian degeneration of normal nerve fibers, and this cannot be explained purely by the smaller caliber of the regenerated fibers. It is necessary here to consider the increased enzymic activity of the Schwann glia of these fibers, bringing about a more rapid conversion of myelin into fat. Such a hypothesis is in full agreement with the results of I. F. Ivanov's work [5, 6], in which the important role of the Schwann glia in the process of Wallerian degeneration was asserted some time ago.

SUMMARY

The process of Wallerian degeneration and resorption of disintegration products after repeated section of the nerve is much more rapid than following a single nerve trauma. The rate of degeneration and disintegration of regenerated nerve fibers after their division is more rapid in all cases, irrespective of the level at which the second trauma is inflicted – above or below the first scar. By their properties, regenerated nerve fibers greatly resemble the nerve fibers of young animals, degeneration of which after trauma is more rapid than in adult animals. This points to a lower resistance of the regenerated nerve fibers and their increased reactivity.

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AN APPARATUS FOR THE CONTINUOUS RECORDING OT THE VOLUME BLOOD FLOW AND THE PERCENTAGE OF OXYHEMOGLOBIN IN ACUTE EXPERIMENTS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 11, pp. 130-133, November, 1960
Original article submitted December 29, 1959

Apparatus of the "bubble flowmeter" type is widely used for recording the volume blood flow velocity [2, 4, 5, 6, 7].

In addition to the advantages of these types of apparatus (the wide range of measurable velocities, the low percentage of error, the possibility of taking recordings synchronously with other indices) they have several essential defects. The most serious of these is the possiblity of production of a turbulent flow of blood, accompanied by a considerable increase in resistance. The principal points at which the laminar movement of the blood is disturbed are are the bubble traps.

The contraction of these traps, in places highly complicated, creates a high additional resistance and conditions favoring thrombosis and frothing of the blood.

The relatively large size of the apparatus, thereby making its fixation in the immediate vicinity of the vessel difficult, the complex radioelectronic system and the necessity for constant watch on the air accumulating in the trap complicate the work of the experimenter and make it impossible to carry out the simultaneous recording of the volume velocity of the blood flow in different vascular fields.

These considerations led us to introduce certain modifications into the construction of apparatus of this type.

The modifications were basically as follows: 1) elimination of turbulence of the blood flow; 2) simplification of the construction of the bubble trap; 3) maximal elimination of conditions favoring thrombosis and frothing; 4) doing away with the necessity for constant entry of atmospheric air into the system of the apparatus; 5) facilitation of the continuous recording of the oxygen saturation of the blood; 6) making the work of the apparatus as automatic as possible; 7) considerable simplification of the radio circuit.

The apparatus consists of two main parts: an artificial vascular bed with devices for measurement of the volume, and an independently assembled electrical system.

The measuring system, which is placed directly by the blood vessel, consists of two blood-vessel cannulas (Fig. 2, 1), a glass and a polythene loop, which form the artificial part of the vascular bed, the photoelectric cell of the oxyhemometer (2), vitrified resistors (3), stabilizing the skin temperature, 2,5 v electric lamps, photoresistors (6), an effector relay (5), ejecting the air bubbles into the blood stream, an air reservoir of volume 2,5 cc with valves (4) and a bubble trap (7). All the components are mounted on a plastic base, measuring

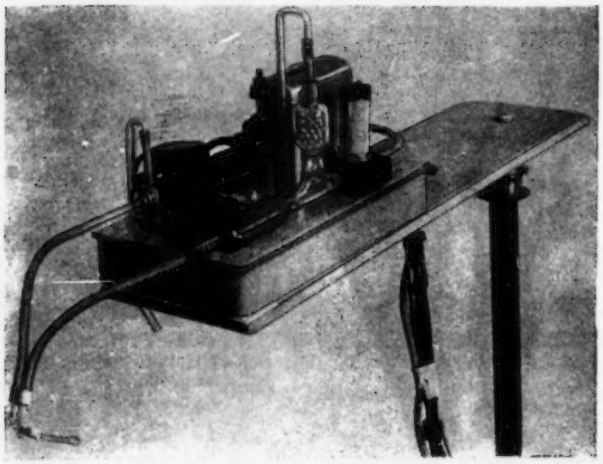

Fig. 1. General view of the recording part of the apparatus for continuous recording of the volume velocity of the blood flow and percentage of oxyhemoglobin in actue experiments.

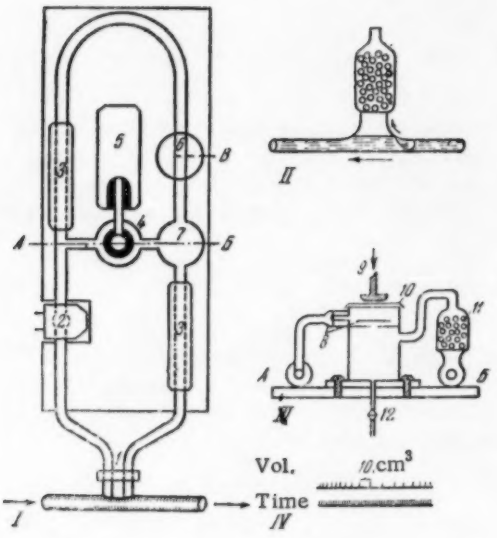

Fig. 2. Diagrammatic scheme of the arrangement of the parts of the apparatus (for explanation see text).

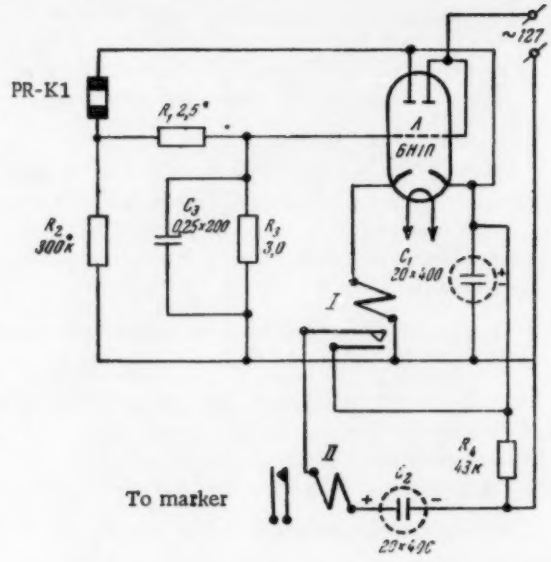

Fig. 3. Principles of the electrical circuit of the apparatus.

9 × 15 cm, which is fixed to a special stand permitting the system to be brought close to any part of an animal's body (Fig. 1).

The principle of action of the apparatus is as follows: blood enters the artificial vascular bed through the entry cannula and passes through an oval enlargement whose internal orifice measures 1 × 6 mm, on which is mounted the photocell of the oxyhemograph (Fig. 2, 2). At the point A the first air bubble is introduced into the blood stream by means of a slight blow from the reed of the effector relay (5). The air bubble, completely filling the tube in cross section, is moved by the blood along the part AB, the volume of which is accurately measured and may be altered at will by the experimenter by replacement by PVC or polythene tubes of suitable length.

At the point B the air bubble passes through a beam of light from the source to the photoresistor (6), as a result of which the effector relay (5) is put into action.

The reed of the relay strikes the membrane of the air reservoir (4) and a fresh air bubble enters the blood stream, isolating the next volume of blood, equal to the first.

Meanwhile the effector relay closes the contacts leading to the marker relay, and a mark is made on the kymograph indicating that a given volume (1, 2, or 5 cc) of blood has passed along the vessel (Fig. 2, IV).

The air bubble, the size of which is controlled at the beginning of the experiment by means of a screw, mounted on the reed of the effector relay, after passing through the photoresistor, enters the bubble trap (7), from which it passes to the air reservoir (4), where it takes the place of the bubble previously expelled into the blood stream.

The initial volume of air is thus maintained and it is no longer necessary to add air constantly from the atmosphere, thus ensuring optimum conditions for recording the oxyhemoglobin concentration.

The mechanism of the continuous circulation of the same sample of air will be more clearly understood from the diagram of a section of the apparatus along the line AB (Fig. 2, III).

At a blow from the reed of the relay (9) on the rubber membrane (10), the next bubble is sent towards the point A from the space between the two petal-shaped valves (8). Its place is accurately taken by an identical bubble from the bubble trap (Fig. 2, II).

As already mentioned, the principal point at which turbulent movement of the blood and thrombus formation develop is the bubble trap. After studies of numerous models of bubble trap in action, we devised the simplest possible variant (Fig. 2, II).

This type of construction permits bubbles to be trapped at velocities greatly in excess of those observed in experimental conditions, does not disturbe the laminar flow of blood and considerably diminishes the possiblity of thrombus formation.

In order to prevent thrombus formation effectively, we used a special silicone coating for the surfaces of the glass and plastic tube (dimethylchlorosilane for glass and aminosilane for plastic) [1].

This coating, which makes the surfac nonwetting, also leads to a considerable decrease in the resistance of the system and to the improved definition of the air bubble, which provides a bettwer light contrast and a sharper shadow on the photoresistor.

To prevent frothing we used the foam suppressor polysiloxane [3]. Glass beads dipped in the foam suppressor, were placed in the ampulla of the bubble trap, and completely prevented the deposition of froth on the valves.

Temperature stabilization was achieved by two vitrified resistors, the magnitude of which was selected by means of electrothermometers so that the temperature difference between the inlet and outlet of the apparatus did not exceed 0.1-0.2° within a range of variation from 32 to 39°.

The electrical circuit of the apparatus was also considerably simplified (Fig. 3).

In this circuit the winding of the positive relay is included in the cathode circuit of the tube. The ohmic resistance of the winding is an automatic bias resistance which compensates for the positive bias voltage arising as a result of the presence of the resistor (PR) between the anode supply pole and the grid of the tube, and it protects the tube from being put out of action if high positive voltages are applied to the grid, as may occur when a high intensity of illumination falls on the photoresistor. The resistor R_4 and the capacitor C_2 control the time of operation of the relay II, the contacts of which are used for the marker.

The modifications in construction which we suggest ensure great accuracy of recording of the volume velocity of the blood flow and a high degree of automatism, so that several such apparatuses may be used simultaneously.

SUMMARY

The authors describe an apparatus of the bubble-flowmeter type. It is superior to other similar instruments since it is capable of removing the turbidity of the flowing blood, has a simplified design of the bubble absorbed

and ensures optimal conditions for eliminating thrombus and foam formation. This instrument has a simplified electric scheme.

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METHODS OF OBTAINING EFFLUENT BLOOD FROM THE ADRENAL GLAND IN CHRONIC EXPERIMENTAL CONDITIONS

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 11, pp. 133-135, November, 1960
Original article submitted March 14, 1959

Changes in the functional state of the adrenals may be demonstrated by many indirect tests, but these tests are inadequate for the more detailed study of their working.

A more complete picture of the function of the adrenals may be obtained by the use of methods of direct determination of the steroid hormones in the peripheral blood of animals. In this case, however, it is essential to bear in mind that the corticosteroids, secreted into the adrenal vein, are utilized quickly by the tissues and, on the other hand, that various metabolites may enter the peripheral blood. Moreover, the low concentration of the hormones in the peripheral blood complicates the estimation of the corticoids secreted by the adrenal gland in small quantities.

The fullest picture of the secretory activity of the gland may be obtained by determining the hormones in the outflowing venous blood. In the majority of investigations carried out in this direction, blood flowing from the adrenals was collected in acute experimental conditions. Operations and general anesthesia, however, are themselves factors known to have a strong effect upon the secretion of corticosteroids, and for this reason many changes in the function of the glands, possibly of importance in the pathogenesis of certain diseases, may remain unnoticed against the background of the operation, or their interpretation may be incorrect. The possiblity of obtaining blood flowing from the adrenals in chronic experimental conditions is therefore of considerable interest, and has already attracted the attention of research workers. As long ago as in 1927, for instance, in experiments on dogs, P. I. Popov [1] introduced an angiostomy cannula by London's method into the vena cava at the point of entry of the lumboadrenal vein. When blood was taken through this cannula, however, the blood entering the syringe was mainly drawn from the vena cava, and it appeared impossible to obtain blood from the adrenals in this way.

The introduction of angiostomy cannulas of the most varied construction directly into the lumboadrenal vein was also unsuccessful on account of the small size of this vessel even in large dogs. A more reliable method was developed by the American workers Hume and Nelson [4] (Fig. 1a). The operation was carried out on a dog. The right lumboadrenal vein was ligated and divided above the adrenal gland. A polyethylene tube was tied into its central end and the tube brought out to the surface. Below the adrenal, at the mouth of the lumboadrenal vein, a clamp was applied in the form of a loop of polyethylene thread. Both ends of this thread were also brought out through the tube.

After suture of the wound, the loop was drawn tight enough to compress the lumboadrenal vein at its point of entry into the vena cava, so that the blood flowing from the adrenals through the cannula tied into the central end of the lumboadrenal vein flowed out to the surface. In the period between taking the blood, the cannula was closed with a cork and filled with heparin solution.

We were unable to obtain blood for more than 4-5 days after operation from animals treated in this manner.

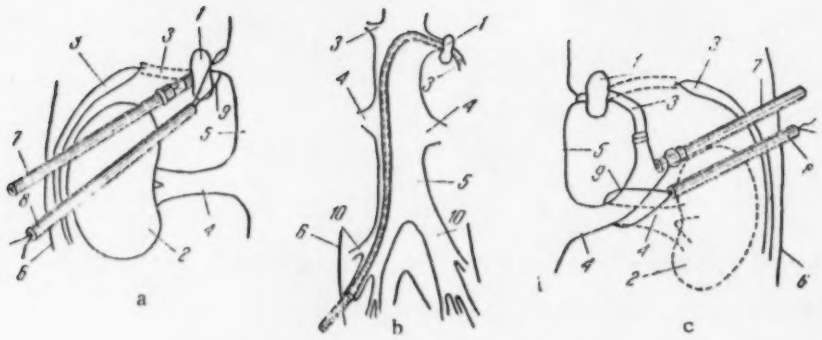

Fig. 1. Scheme of introduction of the cannula. a) By the method of Hume and Nelson; b) by the method of Endroczi et al.; c) after anastomosis of the renal and lumboadrenal veins; 1) adrenal gland; 2) kidney; 3) lumboadrenal vein; 4) renal vein; 5) inferior vena cava; 6) skin; 7) PVC cannual; 8) clamping tube; 9) clamp; 10) femoral vein.

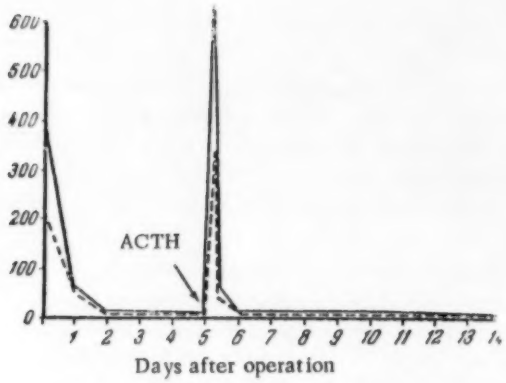

Fig. 2. Rate of secretion of hydrocortisone (continuous line) and corticosterone (broken line) in dogs after insertion of a cannula for the prolonged withdrawal of blood from the adrenal gland.

Judging by the investigations known to us [2, 4-7], it has rarely been possible to obtain blood for a longer time. In our view a drawback of this method is that an "idle" portion of vein is formed between the cannula and the adrenal, and this rapidly becomes obliterated and atrophic.

Another method belongs to the Hungarian workers Endroczi, Bata and Martin [3]. Laparotomy was performed on a dog under general anesthesia, and a thin plastic, elastic catheter was then introduced through the femoral vein into the inferior vena cava (Fig. 1b). The catheter was passed along the inferior vena cava into the orifice of the lumboadrenal vein as far as the point where it received the adrenal veins. Fixation of the catheter in the required position was achieved by attaching it to the inner wall of the vena cava by means of a few fine silk sutures. The wound was closed. When the dog had recovered from the operation, the adrenal blood was drawn off as required through the external end of the catheter. In the intervals between taking the blood samples, the catheter was closed with a cork and filled with heparin solution. According to the authors of this method, the catheter may remain in the animal for 2-3 weeks. When blood is taken by this method, it is evidently possible for blood to be aspirated from the vena cava. If the blood is collected at a time of intensive secretory activity of the glands, this does not matter very much, for in calculating the rate of secretion it is important only that all the blood flowing from the adrenal gland during a known time should be obtained, and its dilution with peripheral blood is of no consequence. In those cases, however, when the gland is in a resting state and the secretion of corticosteroids is very slight, the dilution of adrenal venous blood with peripheral blood becomes undesirable. In our work, we have therefore, attempted to modify the method of Hume and Nelson in order to prolong the time during which adrenal blood may be obtained. Our suggested method is as follows (Fig. 1c).

The operation is carried out on large dogs (weighing 20-25 kg) on the left adrenal gland. After division of the abdominal muscles along the posterior edge of the last rib, the kidney is exposed. The ureter and renal arteries are ligated and divided. The renal vein is mobilized as far as its point of ramification. Ligatures are applied to both branches of the vein. The kidney is removed. The lumboadrenal vein is next dissected out. All veins entering it are ligated. The vein itself is ligated and divided above the adrenal. By means of an

apparatus for vascular suture the central end of the lumboadrenal vein is sutured to one of the branches of the renal vein. Into another branch is tied a polythene tube so that no "idle" areas of vein are left. The tube is brought out to the surface. The mouth of the lumboadrenal vein is ligated and to the mouth of the renal vein is applied a clamp of high-number "saturn" wire. By this method of introduction of the cannula, no "idle" areas of vein are formed. Throughout its whole extent, the walls of the vein are bathed with flowing blood. The operation is carried out at some distance from the adrenal, so that the gland remains free from trauma. The animals withstand the operation well. From dogs undergoing operation in this way, adrenal blood can be collected for 10-15 days after operation. In Fig. 2 the results showing the secretion of corticosteroids in one dog undergoing operation by this method are illustrated. It may be seen from Fig. 2 that the rate of secretion of both hormones – corticosterone and hydrocortisone – fell to a low level on the day after operation, and remained there throughout the period of investigation. The injection of 40 units of ACTH caused a sharp but transient rise in the secretion of both corticoids, with a maximum one hour after the injection of ACTH. It can be concluded from these findings that the adrenal function in dogs undergoing operation by the method described above rapidly returns to normal, and that the reaction of the glands to injection of ACTH is not affected.

SUMMARY

The author describes a method of a cannula implantation for procuring blood flowing out from the adrenal gland in chronic experiments on dogs. The method involves nephrectomy, uniting the lumboadrenal vein to one of the renal vein branches and introduction of polyethylene tube into another branch. This method permits blood to be obtained from the adrenal glands for a period of 10 to 15 days after operation.

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RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

*BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

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Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyateinosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

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1959 cloth 256 pages illus. $7.50

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CHAPTER I. CAUSES OF CORROSION CRACKING OF METALS
Effect of factors relative to the structure and properties of metals and alloys and diffusion processes taking place in metals and alloys; effect of industrial treatments on metals during manufacturing of finished products; effect of the surface state; effect of the nature, concentration and temperature of the aggressive medium; effect of the nature, amount and distribution of stresses; mechanism of corrosion cracking of metals and alloys; conclusions.

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(up to 10% of alloyed elements)
Effect of the composition and structure of the steel; effect of the nature of the aggressive medium; conclusions.

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Chromium Steels: Intercrystalline corrosion of chromium steels; corrosion of chromium steels under static stress.

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Aluminum and aluminum alloys; copper and copper alloys; nickel and nickel alloys; magnesium and magnesium alloys; lead; titanium; cracking of other metals; conclusion.

CHAPTER V. METHODS OF PROTECTION AGAINST CORROSION CRACKING
Protection of stressed metals against corrosion cracking; methods of protection of metals against intercrystalline corrosion; conclusions.

CHAPTER VI. TESTING METHODS
Methods of testing resistance to corrosion under stress; methods of testing susceptibility to intercrystalline corrosion; conclusions.

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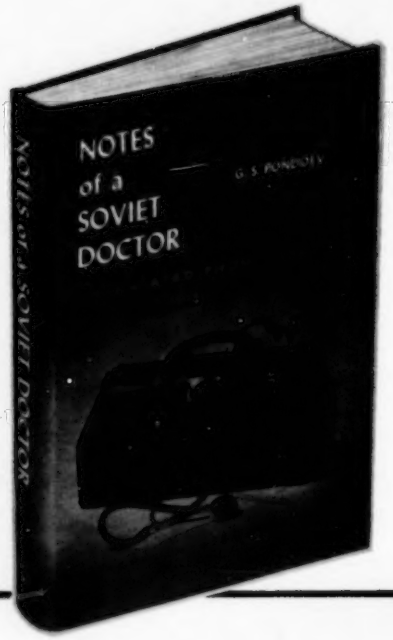

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